THE PATHOLOGICAL ANATOMY OF HYDRAZINE POISONING.[1]

By H. GIDEON WELLS.

(*From the Pathological Laboratory of the University of Chicago.*)

PLATE XXVII.

In a recent article in the *Journal of Biological Chemistry*[2] is reported a study of the influence of hydrazine upon the intermediary metabolism in the dog, by Professor F. P. Underhill and I. S. Kleiner, in which it is shown that although this poison produces extensive anatomical alterations in the liver of dogs, yet it causes little change in the partition of urinary nitrogen and sulphur, beyond that due to the starvation from which animals poisoned with this drug suffer as they always refuse food while under the influence of the drug. During these studies tissues from the poisoned dogs were kindly placed at my disposal by Professor Underhill for histological examination, and I also poisoned and examined the tissues of a few other dogs, as well as of cats and guinea pigs. A summary of the results of this histological study has been included in the article mentioned, but it has seemed that the histological changes observed are so remarkable and of such importance that they deserve a somewhat more extensive description and consideration.

Hydrazine, $NH_2 — NH_2$, is not a substance that is likely to become so commonly used that it will be of general toxicological importance, although the phenyl derivative, phenyl hydrazine, has long been known to have sometimes had poisonous effects upon those who have used it extensively in studying sugars by the methods devised by Emil Fischer. However, the lesions produced by hydrazine are of such a nature that this drug promises to have a field of usefulness in certain metabolic and pathologic investiga-

[1] Received for publication April 27, 1908.

[2] *Jour. of Biol. Chem.*, 1908, iv, 165.

tions. In these experiments the sulphate of hydrazine has been used, and the general effects are described by Underhill and Kleiner as follows: "The researches of Borissow, of Pohl, and of Poduschka have demonstrated the relatively great toxicity of this compound, and have defined the series of manifestations following its introduction into the body. With doses of 0.1 gram of hydrazine sulphate per kilo of body weight subcutaneously injected, vomiting is observed, which is succeeded by extreme restlessness. There is augmentation of the heart beat which later falls far below the normal, and respiratory difficulty is accompanied by general paralysis. At this stage a short period of coma usually ensues which terminates in death. The entire cycle of events is completed within a very few days. Coincident with the symptoms noted above is the appearance in the urine of variable quantities of protein and bile pigments, together with appreciable amounts of allantoin crystals. The liver appears to suffer fatty metamorphosis, and autolysis of various tissues of such experimental animals leads to the presence of significant quantities of allantoin in the digestion mixtures."

Organs from eleven dogs, two cats and four guinea pigs poisoned with hydrazine have been studied, and the anatomical changes resulting have been found to be quite constant. The poison is remarkable in that it affects only the liver, so far as the histological evidence shows, leaving all the other organs unaffected. In the liver the changes are very constant, although the degree of change has not always been found to vary directly with either the size of the dose or the duration of the poisoning, presumably because of individual variations or inconstant conditions in the dogs examined. The changes consist essentially in a fatty degeneration of the cytoplasm of the liver cells, which begins in the center of the lobules and progresses outward, until in dogs dying from three to six days after being poisoned the process has extended to involve extensively all but the peripheral cells of each lobule, and no cells are present that do not contain more or less visible fat. Only the cytoplasm of the cells is primarily affected by the poison, the nuclei remaining intact until long after the cytoplasm has become a barely distinguishable network of protoplasmic granules and threads inter-

spersed between closely packed droplets of fat; when the degeneration has reached this degree the nucleus may begin to stain faintly, enlarge, become vacuolated, and disappear, as if suffering from lack of nutrition on account of the quantity of fat surrounding it. After a time the cells in the center of the lobule lose not only their cytoplasm, but the fat that has replaced it also disappears, so that nothing remains of the liver cells in the center of the lobules but an occasional badly degenerated cell or a practically naked nucleus; the place of the cells is usually taken by compensatory dilatation of the capillaries. Often there is some fatty change in the epithelial cells of the bile ducts, but the stroma cells and the stellate cells of Kupfer are entirely unaffected. Occasionally there are present in the bile vessels homogeneous bile-stained plugs. There were never observed instances of primary destruction of the nucleus, and the features of the ordinary central necrosis of the liver produced by bacterial poisons are entirely lacking; nothing like focal necrosis has been observed, and there is little or no tendency for leucocytic accumulation in the liver.

When stained with Sudan III, the vacuolization of the liver cells is seen to be due entirely to fat, which accumulates as minute droplets, packing the cytoplasm of the most affected cells so full that it seems to be one mass of fat. The nucleus of even the most fatty cells usually appears unaltered, and retains its position in the cell, surrounded on all sides by fat. In all marked cases there are practically no liver cells that do not show more or less fat, but the demarcation between the greatly affected central portion and the less affected peripheral portion is usually quite sharp, because of a sudden transition from cells packed with fat to cells containing but a few droplets. Fat droplets are also found frequently in the epithelium of the bile ducts, but not in the cells of the stroma and blood vessels. In those cases in which the effect of the poison has been most marked, so that the parenchyma cells in the centers of the lobules have disappeared almost entirely, the fat will be found occupying the intermediate zone of the liver lobule, the cells of the vessels and stroma left in the center of the lobule being practically free from fat, and the amount of fat in the peripheral cells being small in amount in contrast with the middle zone; in such speci-

mens the histological picture is very striking, the orange Sudan III stain forming a solid band, surrounded on each side by the hematoxylin-stained nuclei of the surviving liver cells.

Hydrazine does not seem to affect organs other than the liver, although occasionally minute hemorrhages into the lung or a small amount of bloody pulmonary edema can be found. In one dog considerable hemorrhage was found in the medulla of one adrenal, and in the same dog there were numerous small interstitial hemorrhages into the pancreas with several typical foci of fat necrosis in the immediate vicinity of the organ. The lungs and kidneys are usually congested, but show no microscopic changes. In most of the dogs the brain, heart, gastro-intestinal tract, pancreas, adrenals, lymphatic system and generative organs have been examined without any changes whatever being found. Fatty changes were never found in the myocardium or kidney by special staining. Apparently hydrazine does not affect the red corpuscles, for the liver cells and the spleen and lymph glands were quite free from abnormal pigmentation. Hyaline thrombi or fibrinous deposits were never observed in even the most altered livers.

The general features of hydrazine poisoning may be shown by giving a typical protocol, as follows:

Dog A. Weight 10.83 kilos; very fat, part pug, male. January 15, 11 A. M., I injected 0.4 grm. hydrazine sulphate in 20 c.c. water, subcutaneously in left flank. The dog was a trifle indisposed the next day, but recovered by the end of 48 hours. January 18, 11 A. M., I injected 0.8 grm. hydrazine into the right flank. At this time the dog seemed perfectly well. January 19.—The dog was found dead in the cage, having apparently died about 6 A. M.

Autopsy.—Subcutaneous tissue at the site of injection is pink, but shows no evidence of infection or acute inflammation. Cultures from this point and from the heart's blood gave no growth. *Liver* was very yellow, friable in consistence, and weighed 335 grams. The centers of the lobules were red, but the rest of the lobule was strikingly yellow. The veins were full of blood. *Heart* showed no evident changes; the cavities were full of dark blood which was not coagulated. *Spleen* showed no gross changes. *Kidneys* were very much congested, with the cortical vessels particularly injected. *Stomach* showed some slight congestion of the mucosa, but no other changes were found here or in the rest of the gastro-intestinal tract. *Lungs* contained a great amount of blood but showed no definite areas of hemorrhage or consolidation.

Histological Examination.—*Liver.* The central part of each lobule when stained with hematoxylin and eosin shows a great decrease in the intensity of staining, this condition involving fully four fifths of the length of the cords

in most cases; in some lobules only a few cells at the corners remain approximately normal. This change affects almost solely the cytoplasm, the nuclei staining with nearly as great intensity near the center as at the periphery. The cytoplasmic change consists of a high degree of vacuolization which reduces the cytoplasm to a coarse network, enclosing vacuoles in size varying from droplets as large as the nucleus down to minute granules; the affected cells are somewhat increased in size. There are no cells, even at the corners of the lobules, that do not show some vacuoles. There can be found no leucocytic accumulation, no congestion, and no connective tissue proliferation. In the central area the liver cells are entirely absent, except for occasional extremely degenerated cells with but minute shreds of cytoplasm remaining; there also are many nuclei that resemble liver cell nuclei, some of which stain faintly and some appear normal. Sections cut in agar, and stained by Sudan III and hematoxylin, show each lobule to consist strikingly of three zones of about equal width. The central zone contains neither liver cells nor fat droplets that are readily recognized, but consists chiefly of surviving stroma and capillary cells, with an occasional degenerated liver cell or liver cell nucleus. The intermediate zone is extremely fatty, rather sharply defined from the central zone, and less so from the peripheral zone. Here the cytoplasm of practically every liver cell is so densely packed with fat that the individual granules cannot be distinguished; but no matter how fatty the cell the nucleus is almost always clear and well stained. Occasionally the droplets fuse and force the nucleus to one side, but more often it is central and surrounded with fat. The peripheral zone differs solely in that the fat droplets are of smaller size and less abundant, so that much of the cytoplasm can be distinguished. Some of the small bile ducts contain fat droplets in the epithelium.

Kidney shows considerable congestion, especially in the pyramids but there are no changes in the epithelium. No fat droplets are found in sections stained by Sudan III.

Lung shows many of the alveoli full of blood, which contains no fibrin and no excess of leucocytes.

Myocardium shows no changes. No fatty changes present in sections stained with Sudan III.

Adrenal shows extensive hemorrhage in the center of the medulla, but no leucocytic accumulation and no thrombosis.

No changes found in any of the other organs and tissues.

When the animals are given enough hydrazine to make them quite sick, and are then allowed to recover, the process of repair of the liver lesions seems to consist simply in a gradual decrease in the amount of fat in the cytoplasm until the cells resume their normal appearance, which requires two or three weeks. The cytoplasm of such cells has been found extensively vacuolated, but not reacting for fat, so that there is apparently some intracellular edema following removal of the fat. This recovery of greatly degenerated cells shows how specifically the poison affects the cytoplasm

without injuring seriously the nucleus, and without destroying the vitality of the cell. No evidence of regenerative proliferation of the liver cells and bile capillaries, or connective tissue overgrowth, which are so characteristic of the recovery from acute yellow atrophy, has ever been observed.

Summary.—Hydrazine seems to be a poison with an almost specific effect upon the cytoplasm of the parenchymatous cells of the liver, for when the poison is given subcutaneously this tissue alone shows evident structural alterations, although equal or greater amounts must reach other organs and tissues. It seems to have remarkably little effect upon other than hepatic cells, and does not cause any appreciable destruction of red corpuscles; slight hemorrhages are occasionally produced, but much less than by other poisons with a similar effect upon the liver. It attacks only the cytoplasm of the liver cells, never affecting the nucleus primarily, and causes a profound fatty metamorphosis of the type commonly referred to as "fatty degeneration." In this respect it resembles phosphorus, from which it differs in two important particulars. Hydrazine attacks first the cells in the center of the lobules, while phosphorus shows its first and most marked effects upon the peripheral cells; and secondly, phosphorus usually causes marked fatty changes in the myocardium, the kidneys, and indeed throughout the body, whereas the effects of hydrazine seem to be limited almost absolutely to the liver. The unknown poisons of acute yellow atrophy and eclampsia, and most of the bacterial poisons, attack first and chiefly the nuclei of the liver cells, in contrast to the strictly cytoplasmic effects of hydrazine. Phosphorus also affects the nuclei more than does hydrazine. On this account the recovery of the liver to normal after hydrazine poisoning is remarkably rapid and complete, there being no permanent anatomical alteration after recovery from a most severe non-fatal poisoning.

As a poison for use in experimental studies of hepatic metabolism, hydrazine would seem to commend itself over phosphorus on account of its more selective action upon the liver. In the maximum sublethal doses given for experimental purposes it will destroy fully as large an amount of liver tissue as will phosphorus; but there will always be left a considerable amount of liver tissue in a fair

state of preservation, and presumably functionally competent, whichever poison is used. When recovery of the experimental animal is desired the return of the liver to normal will probably be more rapid and more complete after hydrazine than after phosphorus.

To the pathologist the peculiar action of hydrazine presents many interesting problems. In the first place, why is it that hydrazine shows its effects first in the center of the lobules, while so similar a poison as phosphorus attacks first the periphery? Again, why does hydrazine act so specifically upon the liver cells, even when subcutaneously injected? And why does it limit its action so specifically to the cell cytoplasm, leaving the nucleus practically uninjured? It seems probable that the use of this drug in studies of fatty metamorphosis might throw light upon some of the obscure phases of this puzzling subject. As to the significance of the effects of hydrazine for physiology I may quote from Underhill and Kleiner, as follows: "The most striking feature of the action of hydrazine upon the animal body is the absence of abnormal relationships in the principal urinary constituents. Yet according to histological examination the liver is profoundly altered in structure and a large proportion of the cells is apparently inactive. The only inference that can be drawn from such evidence is that through the persistence of a small number of normally functionating liver cells this organ is enabled to maintain its intermediary metabolic processes in approximate equilibrium. This is in harmony with the recently published observations of Jackson and Pearce upon the production of artificial liver necrosis by injections of hematoxic sera, and as they have aptly pointed out, constitutes one of the best examples of the 'factors of safety' or protective adaptations in the animal body."

In conclusion, I take pleasure in expressing my indebtedness to Professor Underhill for the opportunity of making this study, and for much of the material that was used, as well as for his permission to publish this phase of the studies upon the action of hydrazine.

EXPLANATION OF PLATE XXVII.

FIG. 1. Showing the fat deposited about the central veins. Stained with Sudan III and hematoxylin, the fat appearing black in the photograph. Condition observed 24 to 48 hours after poisoning.

FIG. 2. Later stage than Fig. 1. About the central veins, shown black in the photograph, are only stroma and liver cell nuclei. The light areas represent corners of lobules; the fat (photographed black) occupies an intermediate zone in the liver lobule, as a band surrounding the relatively unaffected corners of the lobules.

FIG. 3. Same as Fig. 2, but higher magnification. The pale area in the center represents parts of adjacent lobules about a portal radical, which is separated by a dark colored band of fatty degeneration from the central parts of the lobules, where only a few large globules of fat remain.

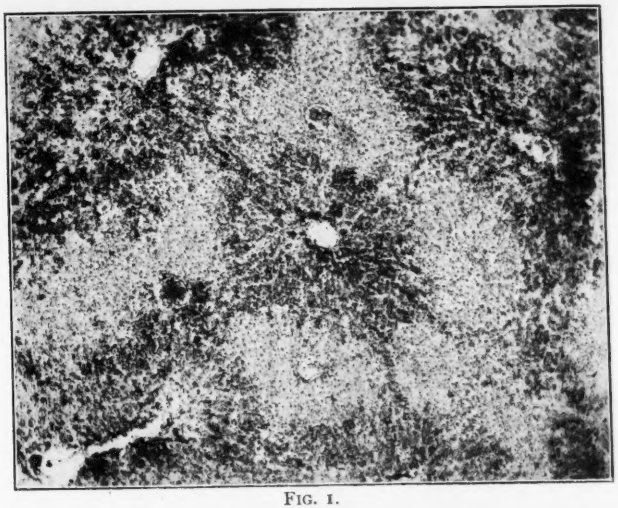


FIG. 1.

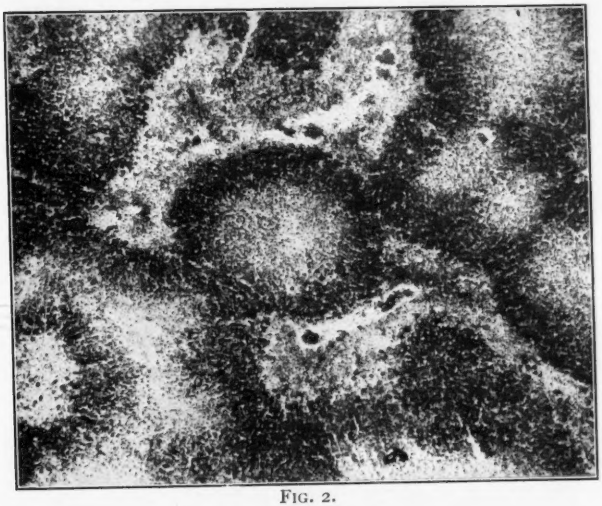


FIG. 2.

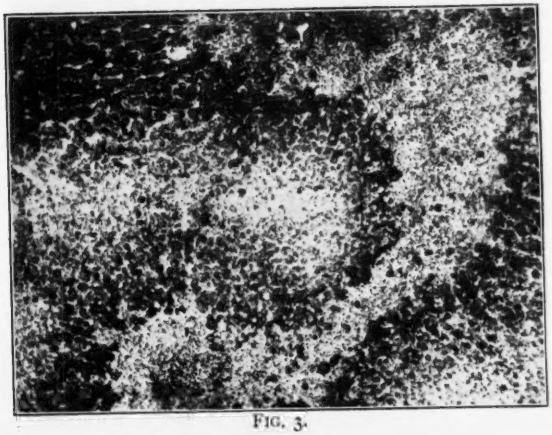


FIG. 3.

MELANOMA OF VATER'S DIVERTICULUM AND LOWER PORTION OF COMMON BILE DUCT CAUSING COMPLETE OBSTRUCTION.[1]

By CHARLES W. DUVAL, M.D.

Director of the Pathological Laboratory of the Montreal General Hospital.
(From the Pathological Laboratory of the Montreal General Hospital.)

PLATE XXVIII.

According to our present conception of pigment-producing cells melanotic tumors do not occur outside the skin, eye and central nervous system, except as metastatic growths. Pigmented tumors that arise in the internal organs are traced in almost every case to a primary growth in a situation where pigment cells normally exist. Most commonly the black moles of the cutaneous surface and the pigment cells of the choroid are responsible for these metastases. Occasionally, however, one cannot find the slightest evidence of abnormal cell activity in the normally pigmented sites, though distant parts show well-advanced melanotic growth. Hence the possibility must be borne in mind that a dislodgment of one or more pigment cells may occur at some time from a normal situation and subsequently become arrested at a distant point. In this way we may account for the occurrence of a melanoma in an internal organ where no primary tumor actually exists.

The neoplasm which is the basis of this study is definitely a melanophoroma and has occurred in the common bile duct of man. The duct was completely obstructed throughout the full extent of the growth. An obstruction of this nature and in this location is, to say the least, unique and aside from its surgical importance affords a most interesting histological study.

A careful search failed to establish the metastatic nature of the obstructing growth. The close resemblance of the cells of this tumor to epithelium renders the microscopic findings of sufficient importance to warrant a detailed description.

[1] Received for publication April 18, 1908.

The clinical history and autopsy protocol are as follows:

Patient, male, white, aged 44, was admitted to the Montreal General Hospital in the spring of 1907, suffering from acute biliary obstruction. The first symptom appeared two months prior to admission as a cutaneous irritation, especially of the skin of the feet; the patient later became jaundiced and jaundice gradually deepened and was associated with great weakness and loss of weight. There was no vomiting at any time.

Shortly after admission a biliary fistula was established and in addition to bile, small granular bodies were intermittently discharged but at no time were definite calculi noted. At the operation a slight cholecystitis but no evident cause for the biliary obstruction was found. Throughout the period of observation the stools were fatty. The patient died early in September, two months after the operation and four months after the first symptoms were noted.

Autopsy was performed fourteen hours post-mortem. The body is that of a white adult male, fairly well developed but emaciated, with marked icterus of the skin, conjunctivae and mucous membranes. Rigor mortis is present. There is slight lividity over the dependent parts. The pupils are unequal, the left being 4 mm. in diameter, the right 2 mm. There is no œdema. In the right hypochondrium is a linear scar 10 cm. in length which traverses the right rectus muscle parallel to the median line. In the line of the cicatrix is a fistulous opening communicating with the gall bladder and on pressure over the upper abdomen the fistula discharges bile. The left parotid gland is the seat of extensive suppuration. Thick purulent material is easily expressed through an existing fistula.

There are no pigmented moles on the surface of the body.

Peritoneal Cavity.—The parietal and visceral peritoneum is deeply stained with bile. The appendix is 12 cm. in length, retrocæcal and twisted upon itself in its outer third. There is no enlargement of the mesenteric or retroperitoneal lymph nodes. The height of the diaphragm is normal. In the region of the gall bladder are numerous old fibrous adhesions especialy about the fundus, formed after its attachment to the abdominal wall.

Pleural Cavities.—The right pleural cavity shows a few old fibrous adhesions over the upper lobe.

Pericardial Cavity.—There is the normal amount of fluid deeply bile tinged.

Heart.—Wt. 215 grm. The subepicardial fat is slight in amount. Pulmonary artery opened *in situ* contains post-mortem clot and fluid blood. The myocardium is of good color and consistence. The endocardium and valves are normal. The aorta and coronaries show nothing remarkable.

Lungs.—In the right upper lobe there is a small calcareous nodule the size of a pea (healed tuberculosis).

Spleen.—Wt. 150 grm. Organ shows nothing abnormal. The gastro-intestinal tract and pancreas are normal. There is a firm gland 1 cm. by 0.5 cm. in the region of the cœliac axis, which on section is deeply discolored with bile, but otherwise shows no abnormality.

Liver.—Wt. 1,580 grm. The lobules are accentuated in outline because deeply bile stained; aside from this discoloration there is nothing noticeable.

Gall Bladder and Ducts.—The inner surface of the gall bladder appears

normal. The fundus is found opened and sutured to the abdominal parietes and communicates through a fistulous opening with the exterior. The cystic duct opened *in situ* is seen to be S-shaped—apparently the result of old adhesions. The wall of the cystic duct is of normal thickness and its inner surface smooth throughout. On opening the common duct it is found to be uniformly dilated to about three times its normal caliber. Its wall is somewhat thinned and the rugæ of the tunica propria present marked fenestration—presumably the result of long-continued distension. *In the lower portion of the duct there is a soft brownish black mass, 2.5 cm. in length, which completely occludes the lumen.*

Kidneys.—Wt. 260 grm. Both organs on section show a dark greenish discoloration of the cortex and medulla. The lining of the pelvis is bile stained and presents numerous small superficial hemorrhages.

The urinary bladder and genital organs are normal.

Brain.—Wt. 1,280 grm. Surface presents nothing remarkable. The pia-arachnoid shows the general deep bile discoloration. Lateral ventricles, pons, medulla, mid-brain and basal ganglia are negative. There is no evidence of abnormal pigmentation. The hypophysis, spinal cord and membranes are apparently normal.

Description of the Tumor.—The growth consists of a soft fungoid brownish black mass 2.5 cm. in length which completely fills the lower portion of the common bile duct, including part of Vater's diverticulum but without encroachment upon the duodenal passageway.

A grooved director was first passed down the lumen of the common bile duct into the gut without meeting the least resistance. Only after the duct had been laid open was the obstruction discovered. The tumor now presented itself as a soft black cylindrical mass which bulged over the edges of the opened duct. It was only with great difficulty that the tumor could be returned to its original place and the severed edges of the duct brought again into apposition.

The tumor throughout its extent was intimately attached to the duct wall and appeared to have started in the tunica propria. There was no thickening of the wall, which seemed abnormally thin. The upper and lower margins of the growth were sharply defined. The base of the growth maintained an even line 2 mm. below the surface epithelium as indicated to the naked eye by the pigment limit. The tumor was confined entirely to the common duct and ampulla. The pancreas and the duct of Wirsung were in no way involved by the growth.

At first it was thought that the tumor consisted of altered blood or inspissated bile; its true nature, however, was discovered after a more careful examination. The melanoma on closer inspection showed innumerable densely arranged flattened finger-like projections which floated free at their distal extremities, but remained firmly attached at their bases as demonstrated by immersion in water.

The tumor with its curious villous structures resembles certain forms of vegetable algæ growing under water. It is noteworthy that no part of the mass could be washed away, nor was the water discolored by the coloring matter of the tumor. On removing the mass from the water the villi immediately collapsed allowing the tumor to again assume a smooth dull black surface.

Microscopic Examination.—Longitudinal strips of the tumor throughout its whole extent were incised for microscopical study. The sections not only included the duct wall, but a part of the pancreas and the surrounding tissues. A portion of the uninvolved duct was included in the sections from the upper and lower limits of the mass in order that the outer cells and their relationship to the normal tissues might be studied. The tissues were fixed in Zenker's fluid, alcohol and formalin, and embedded in paraffin. The material, hardened in Zenker's fluid, was subsequently stained with eosin-methylene-blue for the routine study. Mallory's connective tissue, phosphotungstic-acid-hæmatoxylin and fibroglia stains were employed for the differentiation of special tissues. The material preserved in alcohol was used for the determination of iron-containing pigment; the test, however, was negative.

The growth, in general, under low magnification presents an alveolar structure which is irregularly and loosely formed. In some parts the cells are densely packed, while in others they are loosely arranged without any intercellular supporting tissue.

Sections treated with Mallory's special stains for connective tissue and fibroglia fail to show either of them. As a whole the tumor is without intercellular supporting tissue; an exception to this statement are some alveoli in the periphery of which the lining cells rest upon a delicate basement membrane. This membrane where present consists of fibers which run lengthwise to the glands and contain widely separated flattened cells from which the fibers are apparently derived. In addition to the cell groups which possess these supporting fibrils, there are those which apparently are held together only through cellular cohesion.

I believe that these basement supporting fibers and their cells are the same as those described by Mallory (1) as forming the true basement membrane of adeno-fibromata of the breast. As he points out, these fibrils always accompany the epithelium of the newly formed glands (adenomata) and seem to form as much a part of the tumor as the epithelium.

It is of interest to note that the villous masses comprising the tumor are covered by a thin though well-defined connective tissue envelope, in whose fibrils are elongated cells arranged end-to-end

in an unbroken chain. There is no pigment either intra- or extracellular in this supporting tissue, and in no way does it resemble the melanoma.

The growth apparently started in the gland follicles of the tunica propria where the pigment cells are more numerous within and around the lining epithelium than in any other part of the involved duct wall. Microscopic examination shows that the pigment cells do not extend below the level of the gland follicles, and are so intimately associated with the epithelium of the follicles that it is impossible to distinguish them. This is so striking in some sections that one is inclined to regard the growth as epithelial in origin. Either the normal gland epithelium has become phagocytic to melanin granules of neighboring mesoblastic cells or the epithelium here represents a malignant metamorphosis. Certainly in no portion of the duct throughout the entire basal length of the tumor can one find pigment-producing cells that have involved the vessels beyond the tunica propria.

Many of the glands are composed of single layers of evenly arranged columnar cells that show all grades of pigmentation. Occasionally follicles are lined with two distinct types of cells; the one columnar, mononuclear and non-pigmented; the other bizarre in outline with two or more nuclei and protoplasm completely filled with pigment granules. These two types of cells alternate to form the basal layer of the mucous glands. Possibly these chromatophors should be regarded as foreign cells which have invaded the gland epithelium, since they differ in size, shape and position from the normal columnar cells of the follicles.

These pigmented cells with their long protoplasmic processes frequently alternate with the non-pigmented epithelium, and in places the cell body may be seen pushing its way through the basement membrane of the gland or projecting into the lumen. The processes filled with pigment stream out like tentacles between the normal columnar epithelium. Invariably the protoplasmic prolongations are directed toward the gland lumen; only rarely, indeed, do they point in the opposite direction, though the cell body proper is often beyond the basement membrane or even in the periglandular space.

As a rule the outline of each pigment cell is sharply defined by the brownish black granules, which completely fill every part. The whole is a striking picture of abnormal epithelial activity and suggests that the gland follicle is probably the true generic center of the melanophoroma.

The cells which are free in the lumen of the gland are decidedly angular in outline, especially where they are crowded together. This appearance is even more noticeable in the cells lying free in the alveoli of the melanoma (see Plate XXVIII, Fig. 3). Commonly they are hexagonal, though the chromatophors not associated with an alveolus are round or ovoid. In neither situation, however, are they provided with the protoplasmic extensions which are so characteristic of the pigment cells found in the normal gland follicles. In general the size of the ovoid and hexagonal cells varies greatly, the larger ones measuring 40 to 60 microns and the smaller 4 to 8 microns in diameter.

The new growth is almost entirely composed of pigmented cells; only occasionally are the cells non-pigmented and here apparently they do not belong to the normal tissues. The pigment is most marked in the outer extremities of the villi where the alveolar structure is most pronounced.

The cells have a large distinctly lobulated vesicular nucleus with one or more nucleoli. The nucleolus is always sharply defined and may be very large, sometimes the size of the nucleus of a small lymphocyte. There is often only a narrow rim of nuclear material surrounding an enormous nucleolus. The great variation in size and density of the nucleoli is another prominent feature of the tumor. It is not uncommon to find cells with two or more nuclei held together by narrow bands of basophilic material, each nucleus provided with its nucleolus and normal amount of chromatin.

Mytotic figures are only occasionally found and then in cells sparsely provided with pigment. In certain amœboid cells there may be seen a nucleus in the protoplasmic extension, while a second exists in the cell proper.

Pigment production is apparently in direct proportion to cell activity; the large active cells with deeply staining nuclei containing an abundance of pigment, the small cells with pale nuclei containing

but few granules. The pigment generally occurs in the form of globules arranged in the cell protoplasm equidistant from one another. These globules vary greatly in size, from mere points to that of the ordinary eosinophilic granule. Their color ranges in tint from a light mahogany to a dark blackish brown.

In many cells the pigment granules have coalesced to form one large globular mass which is either centrally or excentrically situated. About such a mass there are apparently as many pigment granules as in the cells of the same size which show no fusion of the pigment. Often the coalesced pigment forms a dense brownish black mass which occupies the inner two thirds of the cell, whilst the periphery is formed by a narrow band filled with light brown granules. Large vacuoles are seen in some cells and undoubtedly represent spaces where the coalesced pigment has dropped out. Many cells are so densely crowded with the granules that the nucleus is not discernible even on change of focus.

The nuclei often contain masses of chromatin-like material which strongly resembles melanin. Whether an excess of nuclear pigment is thrown out and constitutes subsequently the pigment of the protoplasm cannot with certainty be determined.

The pigment differs from that of the melano-sarcoma in that it occurs in the form of evenly arranged intracellular globules instead of irregular scattered masses. The distribution is so regular that it does not seem to be a by-product, but rather an integral part of the cell.

Some chromatophors are phagocytic to others and, in such instances, are easily distinguished from the common endothelial scavenger of blood pigment. The inclusion often fills the greater part of the phagocytizing cell, displacing its nucleus to the extreme periphery. Sometimes there is a broad clear zone about the engulfed cell, outside of which is the protoplasm of the phagocyte.

Occasionally a small vessel in the submucosa of the common bile duct and in close proximity to the tumor contains pigment cells. These cells have the same general morphology and are filled with dark brown granules similar to those of the cells above described. They are not free in the lumen, but appear from their close relation to the intima to be part of the lining endothelium, except that they

are deeply pigmented and bulge into the lumen far beyond the normal cell line. With the exception of these vessels near the tumor the melanotic cells are strictly confined to the main mass. Although some vessels contained chromatophors, contrary to what might be expected, no metastases were found.

A few eosinophile, lymphoid and plasma cells are scattered through the basal portion of the tumor. Below the region of the involved part of the duct there is a well-marked peri-glandular inflammation of lymphoid and plasma cells, but no pigmented cells of any description. Here the cylindrical epithelium of the glands is normal in appearance, though it is evident from the leucocytic infiltration that the follicles are the seat of a chronic inflammation. As far as can be determined all the gland follicles in the tunica propria above and below the growth show a low grade of chronic inflammation, though the other tissues are normal. This fact, in view of the inadequate explanation of the occurrence of this melanoma, strongly inclines one toward Ribbert's (2) theory that there may be some connection between the tumor and this inflammation.

On microscopic examination the pancreas, liver and other organs of the body show nothing remarkable.

Discussion.—The melanophoroma here described is of considerable histopathological as well as surgical interest. We not only have a pigmented tumor unique in its situation, and apparently primary in the common bile duct, but one whose cellular structure resembles epithelium in many ways.

Though secondary growths from melano-sarcoma are common in the liver there is, to my knowledge, no reported case of such a growth occurring in the common bile duct. Statistics show that the gall bladder and ducts are far more frequently the sites of primary non-pigmented sarcomata. In view of this fact the presence of melanin in the tissues where normally it does not exist, as in this case, might impose an insuperable objection to the belief that this is a primary growth. The tumor under consideration, it would seem, originated in the normal cells of the wall of the common bile duct, for the most careful search of the cutaneous surface and central nervous system failed to show anything that would

indicate its metastatic nature. It is to be borne in mind, however, that there is the possibility of a dislodgement at some time of one or more pigment-bearing cells which subsequently have been arrested at the site of growth. In support of this one can advance the recognized metastasis of thyroid cells in remote parts of the body in cases where there is no primary growth in the gland itself.

There are many features of the tumor which suggest an epithelial origin; on the other hand, there are points in favor of its being an alveolar melano-sarcoma. In considering an epithelial histogenesis one is struck with (1) the unusual situation of the growth in a tissue where melanoma have never before been noted, (2) the epithelioid character of the cells and their tendency to mimic in arrangement glandular structures, (3) the intimate association of the cells with those of the normal gland follicles, and (4) the unusual regularity in size, color and distribution of the pigment in each cell, compared with that of the ordinary melano-sarcoma. Of course it is difficult to conceive that a primary melanoma, no matter whether mesoblastic or epiblastic, could arise from cells in this location.

Undoubtedly the strongest point in favor of sarcoma is the presence of melanin, though even here the pigment is atypical in its arrangement and distribution. Ordinarily in melano-sarcoma there are areas that contain no pigment, while others are sparsely supplied and in still other areas pigment is in considerable amount.

If we disregard the pigment for the time, the nature of the growth is only to be determined by its structure and cell morphology. After all the morphological character of the cell is of little value as an aid toward classifying tumors, especially where there is no definite arrangement. The inter- and intracellular fibrils produced by cells certainly offer a more reliable means. In this melanoma of the common bile duct the cells in many parts are similar in morphology to glandular epithelium. They show, moreover, a decided tendency to form alveoli; this fact, considered with the epithelial character of the cells, would make it impossible to class the tumor among the sarcomata were it not for the presence of melanin.

The question naturally arises, can we have a melanophoroma

either meso- or epiblastic in nature which is primary in a situation where normally there are no pigment cells? It is conceivable that epithelial as well as mesothelial cells normally not pigmented may produce pigment under certain conditions.

Hertwig (3) suggests that the pigment of melano-sarcoma is formed from chromatin extruded from the nucleus in an effort of the cell to reduce its nuclear mass, and thus to reorganize itself. In a later paper (4) he describes the transformation of chromidia into pigment. Rössle (5) claims to have traced in the cells of melano-sarcoma the wandering of nuclear substance from nuclei over-rich in this material, and its transformation into pigment in the protoplasm. He suggests that the pigment formation of senile atrophy is produced in the same way as the pigment of actinosphærium and shows, as far as microchemical methods go, the pigments of actinosphærium, melano-sarcoma and brown atrophy to be apparently identical and iron free. Meirowitz-Grandenz (6) in their study of pigment formation in the skin after exposure to light were able to trace both in the nucleus and in the protoplasm pigment from nucleolar substance.

I have observed the formation of autochthonous pigment in corneal epithelium where the cells under toxic irritation become filled with pigment granules. Whether or not this pigment is altered and extruded nucleolar material I am not prepared to say. In consideration of the above facts, I am inclined to believe that the tumor here reported has its origin in cells of the common bile duct.

There is also much evidence in favor of an epithelial histogenesis, especially when we consider that the cells do not produce fibrils or intracellular fibers. Also the more recent work on melanin-producing cells tends to strengthen the conception of their epithelial origin. The pigment may be the result of some peculiar action of certain products of liver metabolism on the mucous gland cells in the common duct, and, as Hertwig suggests, represents the by-product of over-active cells in their attempt at reorganization.

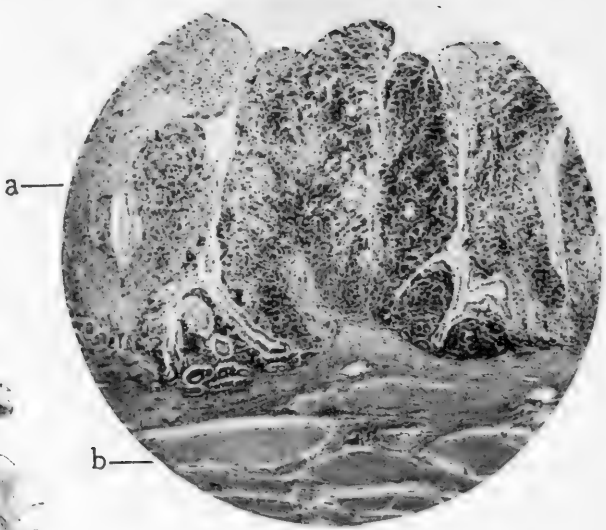


FIG. 2.

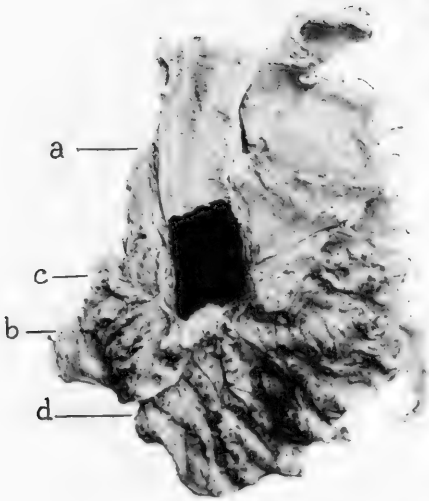


FIG. 1.

FIG. 3.

BIBLIOGRAPHY.

1. Mallory, F. B., *Jour. of Med. Research,* 1905, viii, 113.
2. Ribbert, H., Beiträge zur Entstehung der Geschwülste, 1906.
3. Hertwig, R., *Sitzungsber. Gesellsch. für Morph. u. Physiol.,* 1902, xviii, 77.
4. Hertwig, R., Festschr. für Ernst Haeckel, 1904, 301.
5. Rössle, *Zeit. für Krebsforschung,* 1904, ii, 291.
6. Meirowitz-Grandenz, E., *Monatshefte für praktische Dermatol.,* 1907, xliv, 111, 166.

Explanation of Plate XXVIII.

Fig. 1. Gross appearance of the tumor *in situ.* (*a*) Upper part of the common bile duct. (*b*) Ampulla of Vater. (*c*) Obstructing melanotic growth. (*d*) Duodenum.

Fig. 2. Section through the common bile duct including the melanoma under low magnification. (*a*) Pigmented villous projections which in parts show an alveolar construction. (*b*) Muscularis of the common duct.

Fig. 3. Represents a camera lucida drawing, Leitz, ocular 3, immersion lens 1/16. Note size, shape and arrangement of the pigmented cells.

A BIOLOGICAL STUDY OF THE CEREBRO-SPINAL FLUID IN ANTERIOR POLIOMYELITIS.[1]

By MARTHA WOLLSTEIN, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

During the spring and summer of 1907 several hundred cases of acute anterior poliomyelitis occurred in New York City and vicinity. Towards the end of the summer and in the early autumn a study was made at the Rockefeller Institute of certain of these cases. My part of the study consisted of an effort to find evidence of a micro-parasitic origin of the disease by the employment of the biological reaction of complement deviation according to the methods of Bordet (1) and Wassermann and Bruck (2). This reaction or test has been applied now to the study of a number of infectious diseases, among which are tuberculosis (2), typhoid (3), gonorrhœa (4), and for the identification of particular antigens and antibodies as in syphilis (5) and pertussis (6), with a certain measure of success. The deeper the study is pursued the less convincing has it become that the reaction depends upon specific antibodies in the real sense, and the more probable has it become from the work of Landsteiner, Müller and Pötzl (7) and of Porges and Meier (8) that the lipoids of the blood and organs take an essential part in the reaction. Notwithstanding this, however, it may still display a high degree of specificity.

When this study was begun our knowledge of the conditions of the test was less complete than it is at present. But the methods employed are those still followed, and hence the results of the study are in no way affected by the change of view in respect to the manner in which the complement deviation is produced. The idea from which I proceeded is this: If the disease is due to an hitherto undiscovered microörganism which cannot be detected microscopically, it may still be possible to find evidence of its presence by demonstrating its specific antigen. Since the disease is localized in

[1] Received for publication April 2, 1908.

the spinal cord and brain it was considered not improbable that the antigen might find its way into the cerebro-spinal fluid. By selecting therefore early cases of the disease and performing lumbar puncture this antigenous fluid could be obtained. Having assumed an infectious antigen-producing origin of the disease, the next assumption naturally was that during recovery antibodies to the antigen were formed which were to be discovered in the blood serum and possibly in the spinal fluid itself.

The hypothetical basis for experimental work was thus easily secured and could readily be subjected to a test. For this purpose a number of cases of acute anterior poliomyelitis were available. They were obtained from the Babies' Hospital, the Foundling Hospital, the Hospital for the Ruptured and Crippled, and from the private practice of Dr. L. Emmett Holt. To Dr. Holt and to the house-staff of the hospitals I am greatly indebted for this material. Towards the close of the epidemic an autopsy was secured on a child of two and a half years of age, who had eight weeks previously been admitted to the Babies' Hospital for anterior poliomyelitis and seven weeks later developed symptoms of tuberculous meningitis, to which he succumbed. The microscopic examination showed a localized, characteristic and nearly-healed poliomyelitic lesion in the lumbar part of the cord. Several different tissues from this case were employed in carrying out the tests. The case was not an ideal one for the purpose because of the association of tuberculosis with the other condition, and had the results been positive, they could not have been accepted without confirmation with tissues from pure cases of the disease. In spite of the general negative character of the experiments performed, it is considered desirable to record them on account of their bearing on the etiology of this mysterious disease.

Cerebro-spinal fluid was obtained by lumbar puncture from cases in the second day to the eighth week of anterior poliomyelitis, as follows:

2d day	1 case	11th day	1 case
4th day	2 cases	12th day	1 case
5th day	1 case	3d week	6 cases
6th day	1 case	4th week	1 case
7th day	2 cases	5th week	1 case
10th day	2 cases	6th and 8th week (same case)	1 case

The patients ranged from five months to eight years in age, nine being over two years and four under one year old.

In every instance the fluid was clear and colorless, unless mixed with blood. In about half the cases a small, conical coagulum appeared at the top of the fluid after twenty-four hours' incubation. This did not, in a single specimen, extend throughout the entire length of the liquid. Smears were prepared at once, after centrifugalizing five to thirty minutes, and after twenty-four hours' incubation in the thermostat. The smears were stained with methylene blue and by Gram's method, but cellular elements were entirely absent in fourteen cases. In six cases one to three mononuclear leucocytes were found, but large numbers were never present, and the fluid gave evidence that it was not of inflammatory origin.

Cultures were made on rabbits' blood agar, human ascitic fluid agar, glucose agar, plain agar and in bouillon. Plates were incubated anaërobically as well as aërobically. In fifteen cases the fluid proved to be sterile by both methods. In five a growth occurred. One was identified as a white staphylococcus. One was a short, Gram positive bacillus, and three proved to be a large, Gram positive coccus appearing in pairs and tetrads within large groups. The organism grew abundantly on all ordinary culture media, and is apparently similar to the coccus described by Geirsvold (9) as present in twelve of the cases studied during the Norwegian epidemic of poliomyelitis during the years 1903–06, and again in three of the cases reported by Harbitz and Scheel (10) during the same epidemic. The fluids in my series which contained the diplococci were removed from cases on the fifth day, sixth day and in the fifth week after the onset. In the first two it was present in one specimen of fluid only, subsequent punctures withdrawing sterile fluid. In the third case the coccus was cultivated from a second specimen obtained three days after the first, or on the eighth day of the disease; but not from two later fluids from the same patient. It was looked upon as a contamination.

Geirsvold (9) is of the opinion that the coccus is practically identical with the one described by Jaeger (11) and by Heubner (12), differing materially from the meningococcus of Weichselbaum and from the pneumococcus. He was able to grow it in pure culture

from the blood (number of times not stated) in cases of acute poliomyelitis anterior. Nevertheless he takes a conservative view of its etiological importance, since so little is known of the bacteriological conditions in the central nervous system under normal conditions and in diseases other than poliomyelitis.

Bülow-Hansen and Harbitz (13) found the same organism in one case, their animal experiments proving the cocci to be nonvirulent, as were those isolated from the cases of Harbitz and Scheel (10). Geirsvold (9) had produced atrophy, paralysis, emaciation and death in white mice by inoculating them with cultures of the diplococcus isolated by him.

Schultze (14) looked upon the diplococci he saw in smears from the cerebro-spinal fluid in two cases of anterior poliomyelitis as meningococci, although he could not make them grow. Engel (15) obtained a growth of Staphylococcus albus from the clear fluid of one case. Concetti (16) studied ten cases and found that the fluid was sterile in six of them, while meningococci were present in one and pneumococci in two.

Pasteur, Foulerton and Maccormac (17) studied a case of acute poliomyelitis in a boy thirteen and a half years old. Almost the entire motor system was involved. Four lumbar punctures were made. The fluid, clear at first, developed a grayish sediment of lymphocytes. In the first two fluids a Gram positive diplococcus was found, which appeared occasionally in tetrads and once in short chains, but never intracellularly. The coccus could not be made to grow on any culture medium incubated both aërobically and anaërobically. Intra-cranial inoculation in rabbits was followed in one instance by paralysis on the fiftieth day, and the diplococci were found in the spinal fluid when the animal died on the fifty-third day. A second rabbit inoculated from this one developed the same late paralysis, and the cocci were present in its spinal fluid on the fiftieth day. A third rabbit inoculated with the spinal fluid of the second animal remained well. The authors believe that their case is a sporadic example of the disease which Geirsvold studied epidemically. It is apparent, however, that there is a complete absence of uniformity in the bacteriological examinations of the spinal fluid in cases of anterior poliomyelitis thus far recorded.

Only one of my cases came to autopsy. It was that of a boy two and a half years old, who died of tuberculous meningitis two months after the onset of his poliomyelitis. At the post-mortem examination an area of softening was found in the lumbar cord, extending over three centimeters in length and affecting only the right anterior horn. The case was complicated with a general miliary tuberculosis involving the cerebro-spinal meninges, pleura, lungs, spleen, liver, lymph nodes and intestines. Microscopic examination of the lesion showed it to be in the sub-acute stage with fragmentation and disappearance of the motor nerve cells, none being left intact in the right anterior horn. The blood vessels were congested and there was marked emigration of leucocytes from them. The glia cells had proliferated. In the membranes were small collections of mononuclear cells, apparently early tubercles in which a few epithelioid cells were also apparent. The lesion in the anterior horn was limited there, and did not extend to the posterior horn nor across the median line. Beyond an irregular emigration of leucocytes just outside the lesion the white matter was not involved.

The remainder of the cord was ground up, dried and extracted, as were all the organs, the sciatic nerves and the muscles of the leg,[2] according to the method of Marie and Levaditi (18).

Blood was obtained from the median basilic vein in seven cases, five to ten cubic centimeters being drawn off. Two normal children were bled as controls.

Cultures made from the blood in two acute and early cases were negative, and smears from the blood showed no evidence of abnormal cellular elements nor of any parasites.

Complement binding experiments were made with cerebro-spinal fluids from seven cases, drawn on the second, fourth, sixth and tenth days and in the third, sixth and eighth weeks, the fluids having been inactivated and proven not to be anti-hæmolytic in dilutions of 0.2 cubic centimeter.

The fluid from the most recent case (second day) was tested against the blood serum of cases suffering from the disease for

[2] These muscles were used because Dr. Meltzer had suggested that the paralyzed muscles might be the earliest seat of the lesion.

five days, three weeks and eight weeks respectively. Dilutions of 0.2 to 0.005 cubic centimeter were employed (Table I). There

TABLE I.

Immune Serum.	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti-Hen Serum	Result.
1. 5th day case 0.1	0.05	*1. C. Sp. Fl. 2d day* 0.2	0.05	0.005	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
0.001	"	"	"	"	++
2. 3 weeks 0.1	"	*2. C. Sp. Fl. 2d day* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
3. 8 weeks 0.1	"	*3. C. Sp. Fl. 2d day* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++

was complete hæmolysis in all, no binding of complement being apparent. Results with the blood serum of normal children were identical.

This early cerebro-spinal fluid (second day after the onset) was also tested with fluid from a late case (eighth week), but again no inhibition of hæmolysis occurred.

In four cases the serum was tested against the cerebro-spinal fluid from the same case, as well as against that from cases in more recent and more advanced stages of the disease. The results were uniformly negative, no binding of complement taking place (Table II).

TABLE II.

Immune Serum	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti Hen Serum.	Result.
1. 10th day case 0.1	0.05	*1. C. Sp. Fl. same case* 0.2	0.05	0.005	++
0.05	"	"	"	"	++
0.02	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
0.001	"	"	"	"	++
2. 10th day case 0.1	"	*2. C. Sp. Fl. 2d week* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
3. 10th day case 0.1	"	*3. C. Sp. Fl. 3d week* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++

Extracts made from the medulla, spinal cord, sciatic nerves, liver and tibial muscles were tried with the serum from cases of poliomyelitis in the eighth week, third week, and on the tenth day after the onset, as well as with serum from a normal child of the same age. Complete hæmolysis was the result in every instance (Table III). In combination with the spinal fluid from a case in the

TABLE III.

Anti-Serum.		G. P. Complement.	Antigen.		Hen's Corpuscles.	Anti-Hen Serum.	Result.
1. 6th week	0.1	0.05	*1. Sp. Cord Extract*	0.2	0.05	0.005	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
2. 3d week	0.1	"	*2. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
3. 10th day	0.1	"	*3. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
4. Normal case	0.1	"	*4. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++

eighth week, the results were the same. Varying the amount of antigen from 0.2 to 0.001 cubic centimeter made no change in the resulting hæmolysis. Tests with extracts from the organs of a normal fœtus, similarly prepared, gave identical results.

TABLE IV.

Anti-Serum.	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti-Hen Serum.	Result.
0	0.05	0.2	0.05	0.005	++
0.2	0	0.2	"	0.005	—
0.1	0.05	0	"	0.005	++
0	0	0	"	0.005	—
0	0.05	0	"	0.005	++
0	0	0.4	"	0	—
0.2	0	0	"	0	—
0	0.05	0	"	0	—
0	0	0	"	0	—

All the controls listed in Table IV were made for every series of tests.

It is to be regretted that no autopsy was obtainable in an early and uncomplicated case.

The results obtained show that no two interacting substances, presumably antigen and antibody, capable of uniting and anchoring complement were demonstrable in the blood serum, cerebro-spinal fluids and organ extracts studied. Therefore the diagnosis of poliomyelitis by means of a serum reaction is apparently not possible, and no light could be thrown on the etiology of the disease by this reaction.

BIBLIOGRAPHY.

1. Bordet and Gengou, *Annales de l'Inst. Pasteur,* 1901, xv, 289.
2. Wassermann and Bruck, *Deut. med. Woch.,* 1906, xxxii, 449.
3. Moreschi, *Berl. klin. Woch.,* 1906, xliii, 1243.
4. Müller and Oppenheim, *Wien. klin. Woch.,* 1906, xix, 894.
5. Wassermann and Plaut, *Deut. med. Woch.,* 1906, xxxii, 1769.
6. Meier, *Deut. med. Woch.,* 1907, xxxiii, 1558.
7. Laudsteiner, Müller and Pötzl, *Wien. klin. Woch.,* 1907, xx, 1565.
8. Porges and Meier, quoted by Wassermann, *Berl. klin. Woch.,* 1907, xliv, 1601.
9. Geirsvold, quoted by Harbitz and Scheel, vide infra.
10. Harbitz and Scheel, Path.-Anat. Untersuchungen über akute Poliom. und verwandte Krankheiten, Christiania, 1907.
11. Jaeger, *Zeits. f. Hygiene,* 1895, xix, 351.
12. Heubner, *Jahrb. für Kinderhlk.,* 1902, lvi, 359; 1896, xliii, 1.
13. Bülow-Hansen and Harbitz, *Ziegler's Beiträge,* 1899, xxv, 517.
14. Schultze, *Münch. med. Woch.,* 1898, xlv, 1197; *Ziegler's Beiträge,* 1905, 7th supplement, p. 551.
15. Engel, *Prag. med. Woch.,* 1900, xxv, 135.
16. Concetti, *Rev. Mens. des Mal. de l'Enfance,* 1900, xviii, 550.
17. Pasteur, Foulerton and Maccormac, *Lancet,* 1908, i, 484.
18. Marie and Levaditi, *Annales de l'Inst. Pasteur,* 1907, xxi, 138.

THE PULSE PRESSURE AS AN INDEX OF THE SYSTOLIC OUTPUT.[1]

By PERCY M. DAWSON and LEMUEL W. GORHAM.
(*From the Physiological Laboratory of the Johns Hopkins University.*)

Plates XXIX and XXX.

In a communication made some time ago, one of us (Dawson)[2] called attention to the propriety of considering the pulse pressure (that is the difference between the systolic and diastolic pressure) as an index of the systolic output. In this connection it was stated that ". . . experiments on animals, although not yet completed, have been carried far enough to show" that "the pulse pressure varies with the systolic output. In this series of experiments the pulse pressure as determined with the Hürthle manometer was compared with the systolic output as determined with the cardiac plethysmograph of Henderson, and it was found that on stimulation of the peripheral end of the vagus, in asphyxia, hemorrhage, infusion and so forth the pulse pressure and the systolic output vary together." These experiments have now been completed and the results are presented in the present communication.

METHOD.

Anesthesia.—Each dog received from 0.6 to 1.2 grm. of morphia hypodermically, and an hour later was anesthetized with ether, administered at first with a cone, but later through a tracheal cannula.

Recording.—Simultaneous tracings were obtained as follows:

1. The mean blood pressure in the femoral artery by means of a mercury manometer.
2. The systolic output by means of Henderson's cardiac plethysmograph.

[1] Received for publication March 20, 1908.
[2] *British Med. Jour.*, 1900, ii, 996.

3. The blood pressures in the carotid and femoral arteries by means of two[3] Hürthle manometers.

The recording surface was a Hürthle kymographion and the general character of the tracings obtained is shown in Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX).

RESULTS.

The results of the experiments are so concordant that it has been deemed admissible to present only the protocols of two typical

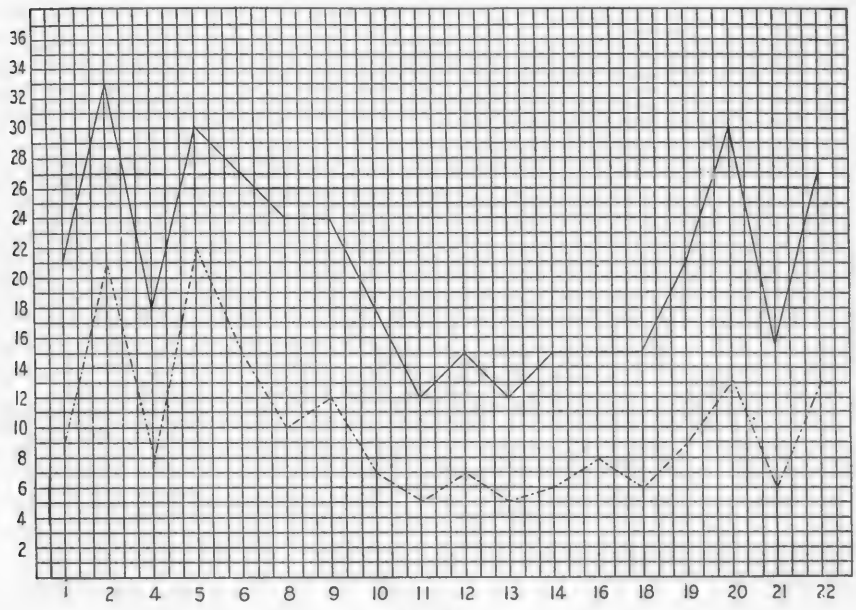


FIG. 1.

experiments. These are given in the form of two tables, two charts and two tracings.

[3] By using two arteries and two manometers we doubled the chance of obtaining an accurate record of the pulse pressure. In Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX), for example, the record obtained from the carotid was not to be relied upon, for after the completion of the experiment it was found on careful examination of the record, that with high pressures the lever pressed too tightly against the paper, while with lower pressures the lower part of the curve was spoiled by an obstruction to the movement of the lever.

Tables.—The numbers in Column I correspond to those which appear running horizontally at the bottoms of Figs. 1 and 2. The figures in Column II have only a relative value. The figures in Column III denote in cubic centimeters the change in the volume of both ventricles. Since most persons conceive of changes occur-

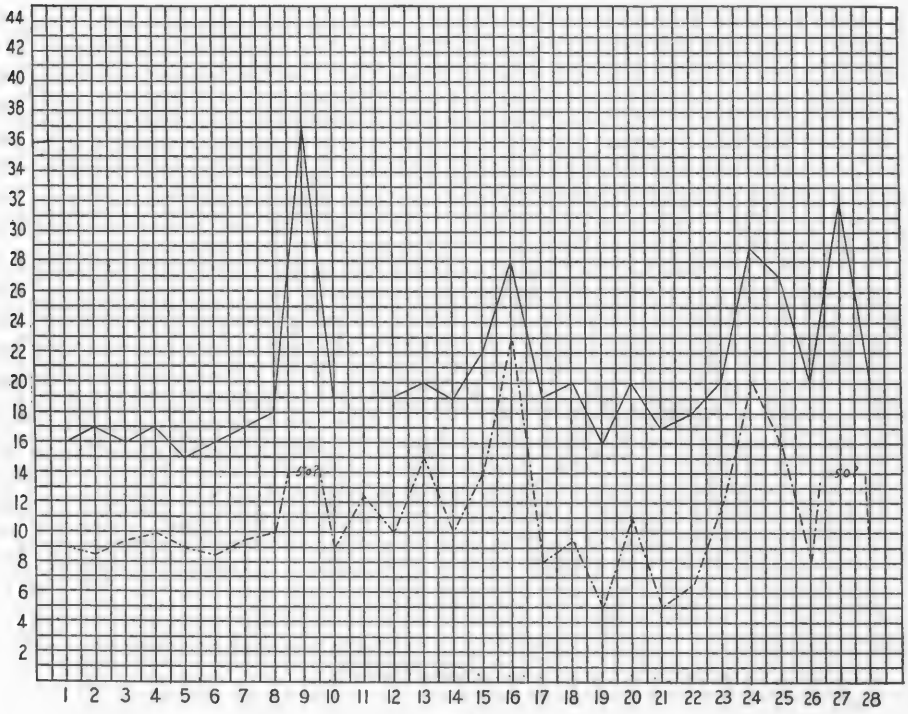


FIG. 2.

ring in the circulation in terms of variations in the mean blood pressure, Column IV has been added to complete the picture. The results given in the tables are also shown in Figs. 1 and 2, which correspond to Tables I and II respectively.

Other explanatory data are the following: asphyxia was produced by stopping the artificial respiration; stimulations were made with the faradic current; the stimulations of the annulus Vieussen-

tis occurred in the course of an ineffectual attempt to reach the accelerators. The carbonate solution had the composition: Sodium carbonate, 7.1 per cent. and sodium bicarbonate, 4.6 per cent.

TABLE I.

Experiment I.

I.	II.	III.	IV.	
Numbers Correspond to Those in the Figure.	Pulse Pressure (Length of Stroke of Hürthle Lever) from Carotid.	Systolic Output (Change in Vol. of Heart in c.c.).	Mean Blood Pressure (Femoral) in mm. Hg.	
1	7	.9	158	After section of both vagi.
2	11	21	60	During stimulation of peripheral end of vagus.
4	6	8	110	After recovery.
5	10	22	54	During stimulation of peripheral end of vagus.
6	9	15	66	During escape from vagus inhibition.
8	8	10	98	Normal.
9	8	12	148	During asphyxia.
10	6	7	100	During stimulation of the central end of the vagus.
11	4	5	54	The same.
12	5	7	78	The same.
13	4	5	136	During stimulation of the annulus Vieussentis.
14	5	6	84	After recovery.
16	5	8	80	After intra-arterial infusion of strong sodium carbonate and bicarbonate.
18	5	6	64	During apnea following the infusion.
19	7	9	90	After a second infusion.
20	10	13	78	After a third infusion.
21	5	6	32	After bleeding.
22	9	13	30	After a fourth infusion.

In this experiment (Experiment I), the record obtained by the Hürthle manometer connected with the carotid artery was the more satisfactory and consequently the values relating to this artery only have been presented. Strong carbonate (see No. 18) usually causes very violent respirations followed by a respiratory pause which usually continues until death unless the animal is kept alive for some time with artificial respiration so that the effect of the carbonate has had time to disappear.

On perusal of the tables, it is seen that the pulse pressure (Column II) and the systolic output (Column III) vary together. This is shown even more clearly in the figures, where the lines which represent the quantitative variations in these two phenomena are seen to be almost exactly parallel.

TABLE II.

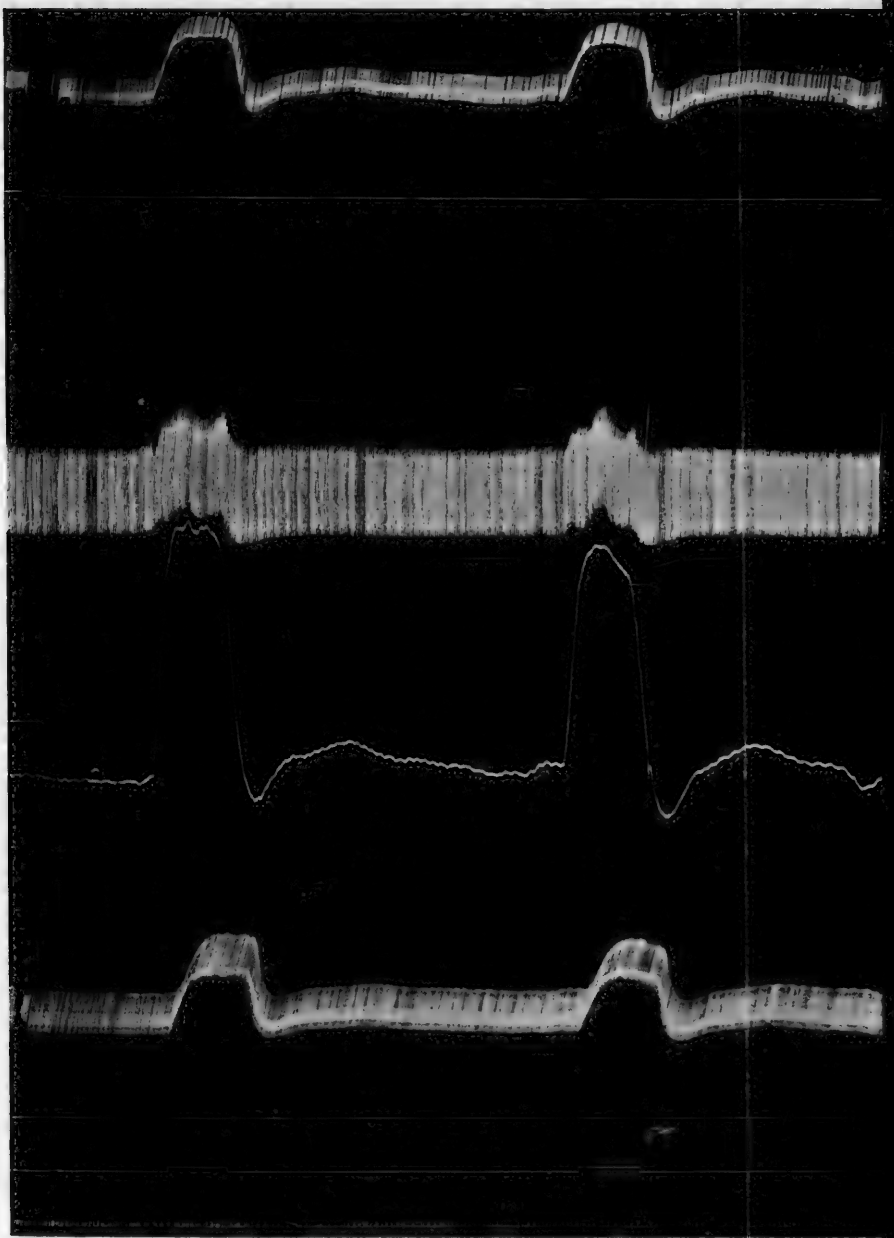
Experiment II.

I. Numbers Correspond to those in the Figure.	II. Pulse Pressure (Length of Stroke of Hürthle Lever) from Femoral.	III. Systolic Output (Change in Vol. of Heart in c.c.).	IV. Mean Blood Pressure (Femoral) in mm. Hg.	
1	16	9	97	Normal.
2	17	8	148	After section of vagus.
3	16	9	139	
4	17	10	228	Stimulation of central end of vagus.
5	15	9	126	
6	16	8	130	
7	17	9	228	Stimulation of central end of vagus.
8	18	10	144	
9	36	50?	52	Stimulation of peripheral end of vagus.
10	19	9	134	
11	19	13	164	Stimulation of the anterior crural.
12	19	10	140	
13	20	17	166	During stimulation of annulus Vieussentis.
14	19	10	130	
15	22	14	220+	Asphyxia.
16	28	28	190+	Asphyxia.
17	18	8	148	
18	19	9	168	
19	16	5	100	Bleeding.
20	20	11	182	Transfusion (intravenous) of 0.7 % sodium chloride solution.
21	17	5	124	Bleeding.
22	18	6	84	
23	21	12	64	Intra-arterial infusion of strong sodium carbonate and bicarbonate.
24	29	21	82	Same.
25	27	16	106	Same.
26	20	8	58	
27	32	50?	54	Intra-arterial infusion of strong sodium carbonate and bicarbonate.
28	20	9	58	

In this experiment (Experiment II), the record obtained by the Hürthle manometer connected with the femoral artery was the more satisfactory and consequently the values relating to this artery only have been given. The very great excursions of the plethysmographic lever (see Nos. 9, 16 and 27) was probably due to the inertia of this part of the apparatus. The curve of the mercury manometer (Nos. 15 and 16) ran into the plethysmographic curve so that the height of the mean blood pressure could not be measured.

CONCLUSIONS.

On the basis of these facts we feel justified in making the following assertion:



1 2

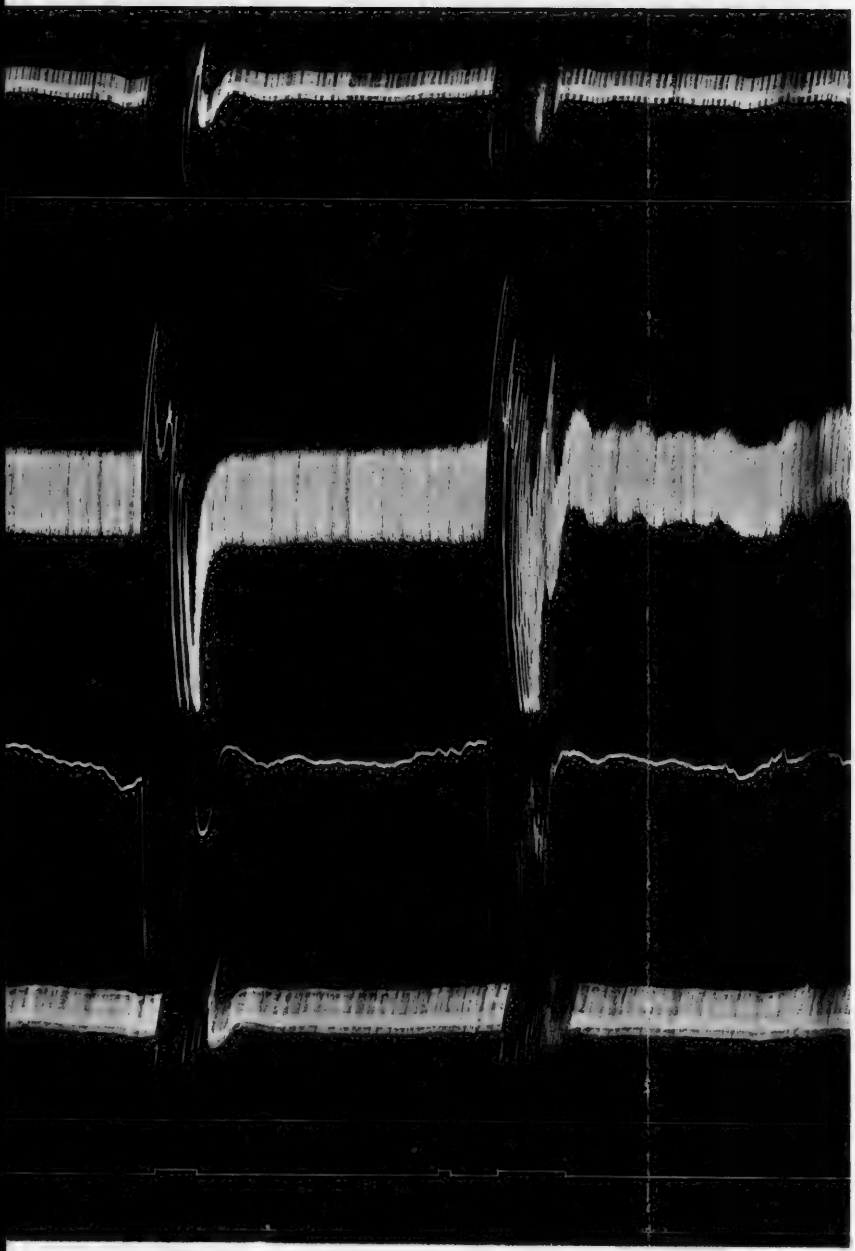


FIG. 3.

PLATE XXIX.

d (Hürtle).

ac plethysmograph.

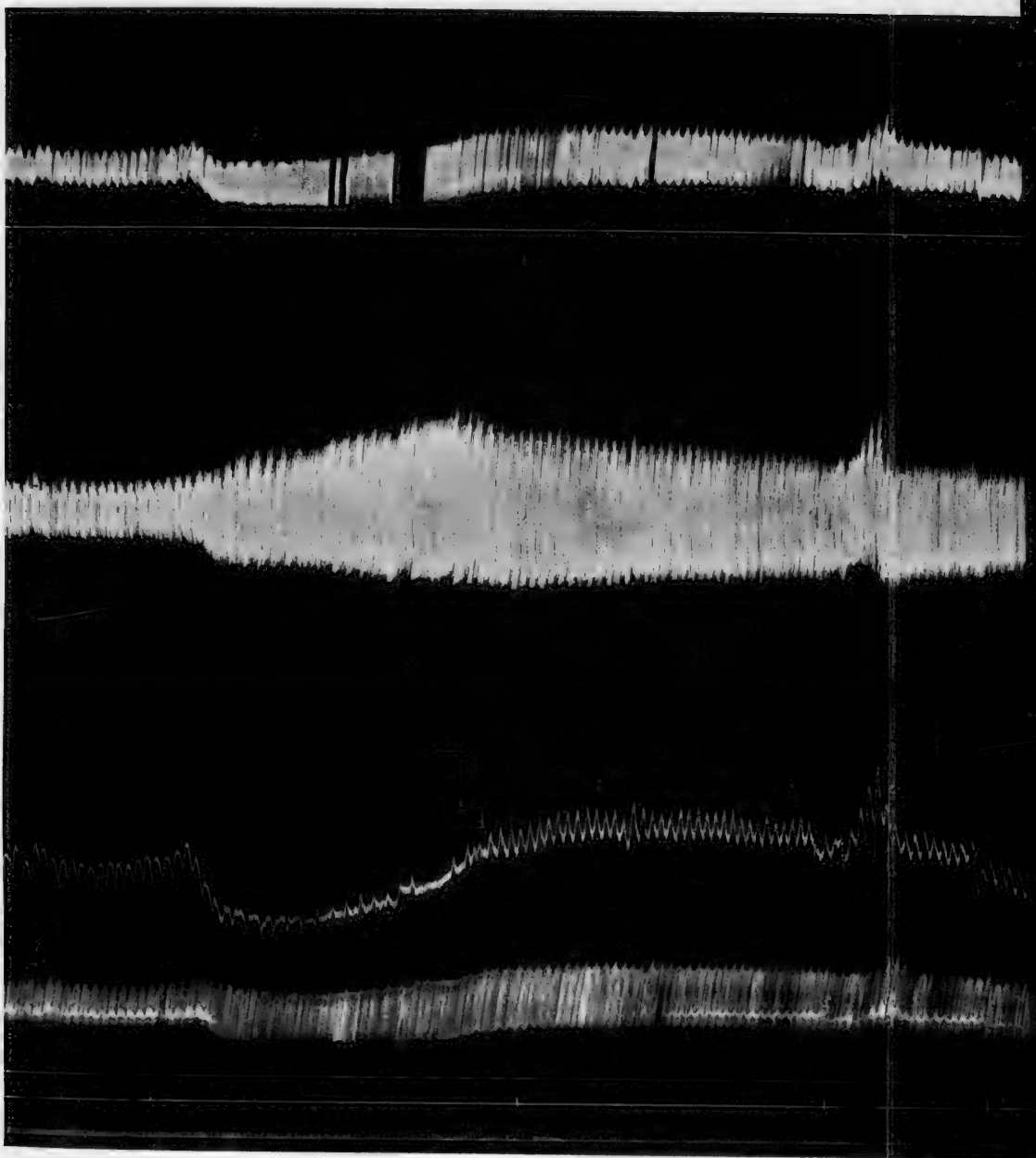
blood-pressure (femoral).

ral (Hürtle).

for Hg. manom. and femoral (Hürtle).

l (stimulation).

ds.



1 2

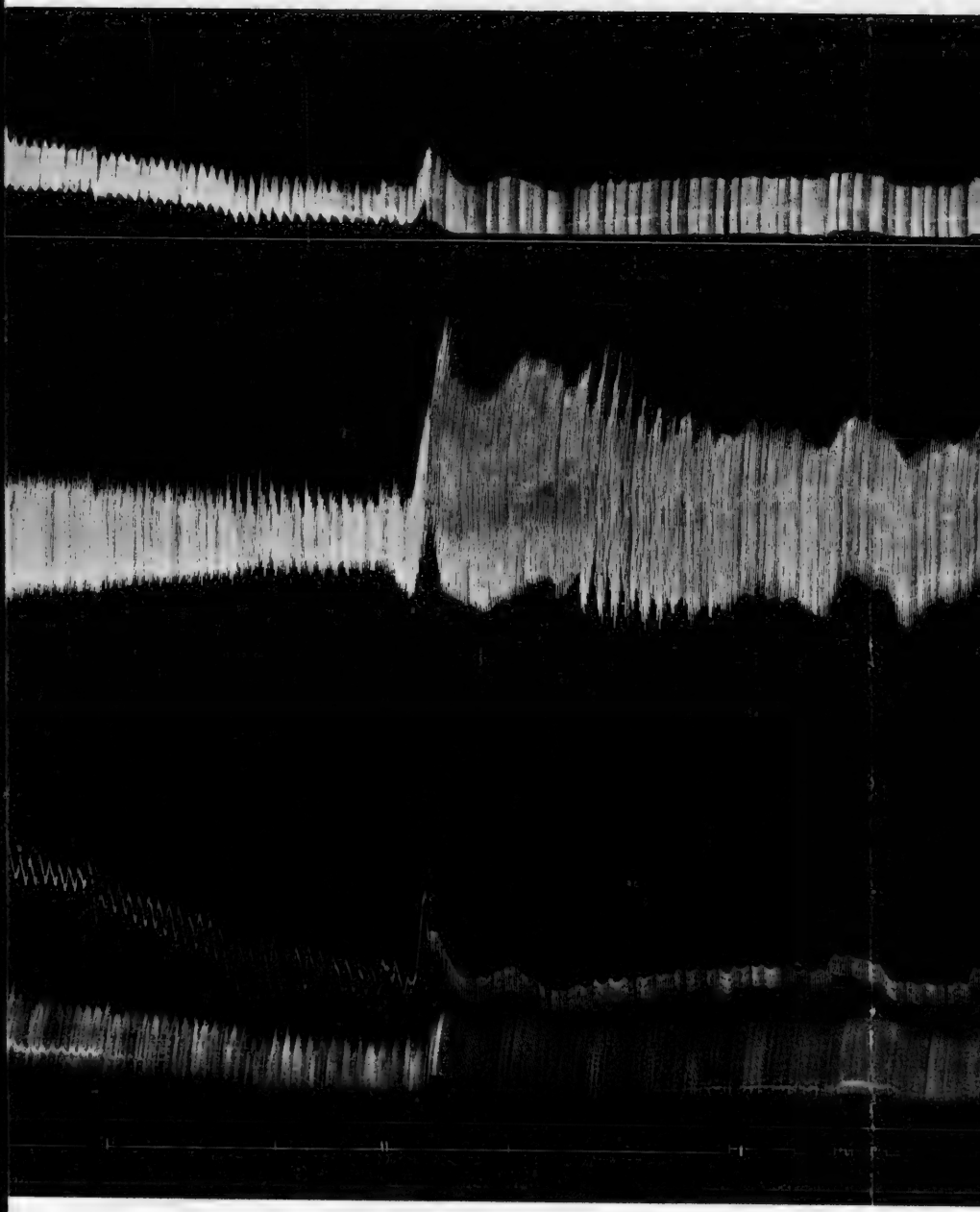
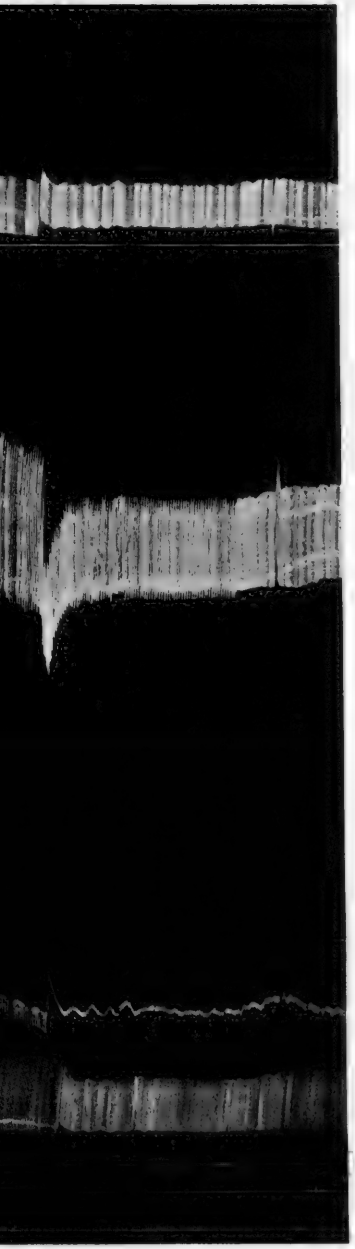


FIG. 4.



Carotid (Hürtle).

Zero.

Cardiac plethysmograph.

Mean blood-pressure (femoral).

Femoral (Hürtle).

Zero for Hg. manom. and femoral (Hürtle).

Under normal conditions and during various procedures (namely, stimulation of the vagus centrally and peripherally, of the saphenus nerve centrally, and of the annulus Vieussentis, intravenous transfusion of 0.7 per cent. sodium chloride solution, intra-arterial transfusion of strong carbonate, bleeding and asphyxia) the pulse pressure is a reliable index of the systolic output.

EXPLANATION OF FIGURES.

Figs. 1 and 2 are diagrammatic presentations of Tables I and II respectively. The solid line represents pulse pressure; the broken line, systolic output. Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX), are parts of the tracing obtained in Experiment II.

PLATE XXIX.

1 and 2. Stimulation of central end of vagus.
3 and 4. Stimulation of peripheral end of vagus.

PLATE XXX.

1 and 3. Intra-arterial transfusion of strong carbonate.
2. Bleeding.
4, 5 and 6. Stimulation of central end of vagus.

STUDIES IN RESUSCITATION: IV. THE RETURN OF FUNCTION IN THE CENTRAL NERVOUS SYSTEM AFTER TEMPORARY CEREBRAL ANÆMIA.[1]

By F. H. PIKE, C. C. GUTHRIE and G. N. STEWART.

(*From the Hull Physiological Laboratory, University of Chicago.*)

Contents.

PAGE.

I. Introduction 490
II. The Rescuscitation of the Bulbar Mechanisms..... 492
III. The Rescuscitation of the Higher Nervous Mechanisms..... 507

I. INTRODUCTION.

The experiments on the resuscitation of the central nervous system were begun more than three years ago. The primary object was to test the possible length of time elapsing after death, after which the return of all the nervous processes could be hoped for, since we had good reason for believing that the central nervous system would prove a weak link in the vital chain, but the further possibility of obtaining new data on some of the nervous processes soon became apparent.

We have described in other papers[2] the main phenomena of resuscitation of the central nervous system after cerebral anæmia. Here we shall briefly summarize the results of the resuscitation of the central nervous system as related particularly to the resuscitation of the entire animal body and incorporate at greater length some new observations on the more strictly physiological phase. Our work has dealt almost entirely with resuscitation after total anæmia except that, rarely, from some slip in technique or, more commonly, for some anatomical reason, the brain and upper cer-

[1] Received for publication April 16, 1908. The previous papers in this series have appeared in the *Journal of Experimental Medicine*, 1908, x, 371; the *American Journal of Physiology*, 1908, xxi, 359, and xxii, 51.

[2] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289; Guthrie, Pike and Stewart, *Amer. Jour. of Physiol.*, 1906, xvii, 344; Stewart and Pike, *ibid.*, 1907, xix, 328; xx, 61; Stewart, *ibid.*, 1907, xx, 407.

vical cord still obtained a small amount of blood. Hill[3] states that "the merest dribble of blood" suffices to maintain a certain degree of activity of the central nervous system for long periods. To this we can fully subscribe. It is possible, also, that the mere presence of blood in the vessels, although completely stagnant, may help to prolong the period of resistance of the tissues to definitive death, so that it may not be indifferent whether the interference with the nutrition of the central nervous system is produced by occlusion of the whole arterial path to the central nervous system, as in most of our experiments, or by simple cessation of the circulation due to stoppage of the heart or, finally, by such a process as strangulation, which occludes simultaneously the arteries and veins. It is obvious that the greatest and most severe anæmia will be caused by the first method, a less complete and more gradual anæmia by the second, while the third will leave the normal amount of blood in the organ practically unchanged. The results of Ségalas d'Etchepare,[4] who found that the struggles of the animal persisted longer after ligation of the abdominal aorta when the inferior vena cava was also occluded than when the aorta alone was tied, would seem to indicate that stagnant blood is better than no blood at all in contact with the tissues. It is possible that such differences account, to a certain extent, for the discrepancies in the literature as to the length of time the circulation in the central nervous system can be stopped without rendering resuscitation impossible.

D'Halluin[5] states that the secondary alterations of the central nervous system following anæmia are not immediately fatal nor without remedy. He does not believe that Battelli's[6] limit of twenty minutes after cessation of the respiration is the absolute one for the resuscitation of the central nervous system, and is inclined to believe that there is experimental as well as clinical evidence that the central nervous system is capable of resuscitation after periods of perhaps relative but very prolonged anæmia. Green[7] states that in one of his cases, a boy nine years old, the

[3] Hill, *Philos. Transactions of the Royal Soc.*, 1900, cxciii, 121.

[4] Ségalas d'Etchepare, *Jour. de physiol. expér. et path.* de Magendie, 1824, iv, 287; cited by Batelli, *Jour. de physiol. et path. générale*, 1900, ii, 443.

[5] D'Halluin, *Presse méd.*, 1904, xii, 345.

[6] Battelli, *Jour. de physiol. et path. générale*, 1900, ii, 443.

[7] Green, *Lancet*, 1906, ii, 1708.

heart was not started by massage until twenty-five minutes after it first stopped, and did not beat strongly until thirty-five minutes after it first stopped. Severe spasms, rapid respiration (40) and pulse (168 in the minute), perspiration and high temperature followed, with death in twenty hours. This case might seem to lend some slight support to D'Halluin's conclusions. We have already pointed out in another place that the time of heart stoppage is impossible to determine except by actual inspection. For this reason we must exclude as evidence many of the clinical cases reported and cited in support of a longer limit, after which resuscitation is possible. We are not inclined, therefore, to give much weight to D'Halluin's contention.

Our experience, on the contrary, is that Mayer's[8] limit of ten to fifteen minutes of total cerebral anæmia, beyond which resuscitation is not possible, is approximately correct. This limit may be extended a little, but not a great deal, where the circulation has stopped completely without causing the extreme anæmia produced by our technique.

The exact moment at which the heart stops beating can be determined by inspection; and the last respiratory movement may easily be observed and recorded. Similarly, in the restoration of these systems to activity, the direct observation of the first movement is not a matter of great difficulty. The determination of the state of the cortical centers is somewhat different. There are, except in the case of the motor cortex in general, no unequivocal and objective signs of the loss or return of function.

In considering the resuscitation of the central nervous system we may most conveniently deal first with the return of the various functions and reflexes separately, considering them as criteria of the physiological condition of their respective centers. We will take up, therefore, the resusciation of the bulbar centers and then the return of the functions of the higher centers.

II. THE RESUSCITATION OF THE BULBAR NERVOUS MECHANISMS.

The Resuscitation of the Extrinsic Cardiac Nervous Mechanism.—The heart continues to beat in cerebral anæmia, apparently little

[8] Mayer, *Med. Cent.*, 1878, xvi, 579.

affected, long after the failure of all the cerebral centers, and even when the vagi, the cervical sympathetic, and the spinal cord are divided. Furthermore, a dog's heart will continue to beat for several hours after the removal of the head. It seems certain therefore, that the mammalian heart will beat without receiving any impulses from the brain. But will the heart beat continue indefinitely in the absence of cerebral impulses? So far, the only experimental answer to this question is that of Friedenthal[9] who severed all the cardiac nerves of rabbits, including the sympathetic, and all of the vagus except the Hering-Breuer fibers to the lungs. The animals lived for several months without suffering any great inconvenience during ordinary activities. We have observed some instances in the course of our experiment which would seem to be incompatible with Friedenthal's conclusions, but the question cannot be discussed here.

Asphyxial slowing of the heart may occur at any time during cerebral anæmia; and even when both vagi and the spinal cord are divided. The heart rhythm after restoration of the cerebral circulation, shown in detail in the tables in our previous papers, may indicate disturbances of the vagus center for several days afterward. Certain other influences, such as hyperthermia, may also enter in to affect the rate, and obscure the vagus effect. Quite apart from these, however, the vagus effect usually returns along with the function of the other bulbar centers, after which it may apparently fail for a time, perhaps several days, and then again finally recover its functional control of the heart.

The question of the nature of the origin of the vagus tone —whether reflex or automatic—has been discussed in a separate paper.[10] In the present paper we will offer some new evidence bearing on this question. We may, for example, investigate the relation between the first appearance of the vagus tone in the resuscitation period, and the first appearance of the vaso-motor tone. If the vagus tone is of automatic or central origin, we would expect a gradual decrease in the pulse rate as the blood pressure rises. As we have pointed out in the first paper of the

[9] Friedenthal, *Arch. f. Physiol.*, 1902, 135.
[10] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 328.

present series, the rate of the heart in the resuscitation period is that of a heart whose vagi have been divided, although the vagus endings in the heart are perfectly active. The heart maintains a practically constant, or even an increasingly high, rate as the blood pressure gradually rises. This evidence, as far as it goes, does not show the existence of an automatic element in the vagus tone. If now we investigate the time relations of the first appearance of vagus tone and the opening up of the reflex pathways for vagus inhibition, we find a very close correspondence in the time of their appearance—that is, there is little or no vagus tone discoverable in the resuscitation period until the integrity of the reflex arc is restored. There is, then, not only no evidence in favor of an automatic or central origin of the vagus tone, but also, as we have shown elsewhere, direct evidence in favor of its reflex origin.

Couty[11] states that the cord constitutes an independent cardiac accelerator mechanism as well as a vasomotor center, modified by anæmia, asphyxia and other influences. Couty used lycopodium spores, injected into the cerebral arteries, to produce anæmia of the brain. In all our experiments in which we were certain that the bulbar centers had ceased to function, we were unable to get the normal increase in heart rate by suddenly lowering the blood pressure. The heart beats on with machine-like regularity during the occlusion period. Barring the possibility of spinal shock in our experiments, we do not consider it probable that accelerator impulses normally arise from the cord below the fourth cervical segment—the lower level of total anæmia in most of our experiments, but we do not deny the possibility that a phylogenetically more primitive mechanism located in the cord, might, under proper conditions, again become active and discharge accelerator impulses to the heart.

One other line of evidence not previously considered remains to be examined, and that is the correspondence in the time of resuscitation of such a mechanism as that for swallowing, which is so evidently reflex, and the return of the vagus tone. But since, as is self evident, the same questions arise in connection with vasomotor tone and respiration, and the same standard of comparison may be

[11] Couty, *Arch. de physiol. norm. et path.*, 1876, Ser. 2, iii, 665.

used in determining their origin, we will first present the facts with regard to these mechanisms and then attempt their interpretation.

The Resuscitation of the Respiratory Nervous Mechanism.—It is obvious that, for the continued life of an animal the resuscitation of the respiratory mechanism is a matter of necessity. The heart may be beating vigorously and the tissues be well nourished so long as oxygen is provided by artificial means, but unless spontaneous respiration occurs, sooner or later death is inevitable. The choice of a method for starting the heart will therefore depend not only upon its efficiency in restoring cardiac function, but also upon the facility with which we may direct a stream of oxygenated blood through the medulla oblongata.

We have considered, in other papers, the resuscitation of the respiratory center after cerebral anæmia in much detail, and we have devoted particular attention to the question of the automaticity of the respiratory center.[12] The resuscitation of the respiratory center after asphyxia and other forms of death is very much the same as after cerebral anæmia provided the period of asphyxia has been reasonably long. There is, in general, the same strong first gasp, and the slow initial rhythm which gradually increases to the normal rate. The time required for recovery from transient respiratory failure due to anæsthesia is, of course, much shorter than that required for recovery from prolonged bulbar anæmia.

The regularity and constancy of the initial respiratory rhythm in resuscitation, three to five a minute, and the similarity of this rhythm to that sometimes observed after section of both vagi, and particularly after section of the higher pathways to the brain, is one argument we have advanced in favor of the automaticity of this center. The further fact that this slow, regular rhythm may persist for a time, the length of which depends partly upon the gravity of the injury to the cells, unaffected by stimulation of the afferent nerves is also an indication that the center is functioning in the absence of afferent impulses—*i. e.*, the center is automatic.

In a small dog (Experiment 1, March 13, 1905)[13] it was noted during the whole time of compression (three minutes and five seconds) of the arch of

[12] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 328.

[13] The experiments are numbered consecutively for convenience in reference. The chronological order is indicated by the dates given.

the aorta and of the innominate artery, that inspiratory acceleration of the heart occurred with each natural respiration, but not with artificial respiration. The impulses discharged from the respiratory to the cardiac center were not, therefore, reflex from the pulmonary fibers of the vagus. The quickening of the heart toward the end of the inspiration has been observed, in our later experiments, at a time when the afferent impulses did not affect the respiration.

Certain other structures participate in the respiratory activity by associated movements, notably among these being the eyelids.

For example, in Experiment 2, after an occlusion of sixteen minutes in a cat, the respiration was going on 34 minutes after the release at the rate of twelve in a minute. The movements were strong and extensive, the whole head participating in them. There were also strong fibrillary contractions of the tongue, and violent twitching movements of the vibrissæ. The pupils were well contracted, but there was no corneal, light or lid reflex. The animal winked its eyes slightly but distinctly, the upper eyelid descending with each respiratory movement. Again, in Experiment 3 (June 7, 1905), 28 minutes after an occlusion of 31 minutes, the pupils were well contracted, and the light reflex had returned, but not the corneal. The respirations at this time were rapid and in groups of four or five, getting progressively stronger up to a maximum. Strong nostril movements accompanied the respiratory movements. The eyelids moved with the strong respiratory gasps, the aperture of the eye widening, but not with the weak gasps.

The synchronism of the natural with the artificial respiration is a peculiar phenomenon seen in some of the experiments. An instance of this occurs in

Experiment 4 (March 29, 1905). Half grown cat. Ether. Tracheotomy. Artificial respiration. Opened chest.

1:40:15 P. M. Clamped aorta just above intercostal branches.

1:41. Clamped innominate and left subclavian arteries.

2:01. Released innominate and left subclavian arteries.

2:06. A gasp.

2:06:20. Another gasp. Gasping respiration goes on regularly, five in the minute.

2:11. No movements on striking forelimb.

2:12. Respiration strong and extensive, eight a minute.

2:14. Respiration 12 a minute, including some small respiratory movements one of which sometimes precedes a deep one. No movement elicited by striking the forelimb.

2:17. No light, lid, corneal, or ear reflexes. The respiration often commences with a rather prolonged, slight opening of the mouth, which ends in a wide gasp.

2:21. Pupil contracted down to a slit. No light reflex.

2:23. No eye or ear reflexes. No movements on pinching ear or on striking forelimbs. Temperature 33° C. in the thorax.

2:28. Swallowing movement.

2:30. The natural respiration is now synchronous with the artificial, ten in 18 seconds, extending to nose, mouth and chest.

2:33. On striking forepaw, get movement of same limb and very slight movement of the opposite shoulder.

2:34. No eye or ear reflexes. Pupils still slits. The natural respiration continues synchronous with the artificial.

2:37. On touching cornea, the eye seems to water, although the eyelids do not move. Marked movement of forelimb on striking it; also movement of opposite forelimb.

2:43. Fair corneal reflex. No light reflex. Strong movement of forelimb when struck and weaker but distinct movement of opposite forelimb.

2:45. Strong light causes marked closing of eyelids but no change in the pupil. Repeated many times.

2:50. Corneal reflex quite marked; also the foreleg reflex, which crosses from either side. Stopped artificial respiration. Cat breathes slowly by itself. Started artificial respiration again.

2:55. The forelimbs are extended and getting stiff. Light reflex is present in pupil.

3:00. Winks eye when ear is flicked. No ear reflex.

3:10. On pinching larynx, get a swallowing movement every time. (Stimulation of superior laryngeal?)

3:15. Stimulated left vago-sympathetic. Causes stoppage of heart, maximum dilation of pupil, bulging of eye and retraction of nictitating membrane.

At times the synchronism assumes a different form.

Thus, in Experiment 5 (March 21, 1905), beginning about twenty minutes after the head arteries were occluded, the cat inspired synchronously with the artificial inflation of the lungs, but only half as often. Twenty-two minutes from the beginning of the imperfect occlusion, natural expiration occurred synchronously with the inflation of the lungs, and exactly half as often. The occlusion lasted, in all, 30 minutes. Fourteen minutes after the release of the head arteries, the artificial respiration was interrupted, and the cat, which had been, for several minutes, breathing synchronously with the artificial respiration, went on breathing for 25 seconds with exactly the same rhythm.

Such instances are too common to be mere coincidences.

Apnœa during the resuscitation period is not uncommon. Instances of this have been given in previous papers. In one experiment, lowering the systemic blood pressure apparently again started respiration. Other cases in which hemorrhage or trephining the skull caused the apnœa to cease, *e. g.*, in Experiment 16, the protocol of which is given later on, have been encountered.

In Experiment 6 (May 13, 1905), three hours after release following an occlusion of one hour, stopping the artificial respiration caused respiratory movements to begin after a considerable interval. These movements involved, at first, the diaphragm alone and later on, the ribs also. Apnœa had lasted two hours.

Well-marked periodic respiration of the Cheyne-Stokes type sometimes occurs well on in the resuscitation period.

In Experiment 7 (May 2, 1905) the first gasp occurred fifteen minutes after a release from an occlusion of 30 minutes. The first gasps occurred at the rate of four in 64 seconds. About four hours after release, the respiration was 140 per minute, with strong associated movements of the nostrils. Pressing the fingers over the chest on each side of the sternum caused marked slowing of the respiration, persisting as long as the pressure was maintained. Twenty-five minutes later, repeating the pressure over the chest caused the same slowing of respiration. The respiratory rate was, at this time (7:38 P. M.), 100 a minute but there were marked variations in the rate at successive intervals of a few seconds. At 8:13, the hind end of the animal was elevated to allow fluid and mucus to run out of the mouth. The respiration decreased to 86 a minute for two or three minutes, but the rate soon rose again. At 8:18, the hind end was lowered again, and the respiratory rate fell to 84 a minute. Then, with the board in exactly the same position, the respiration rose to 100 a minute at 8:32, then to 144 at 8:38, to fall again to 106 at 8:46, and to 84 under the influence of a small amount of ether at 9:00 P. M. An hour later, the respiratory rate was 59 in the minute.

Movements closely approximating spinal respiration have also been observed. One example of this has been given in a previous paper,[14] in which we described also the reflexes simulating respiratory movements so readily obtained through the spinal cord in the resuscitation period following prolonged occlusions. One other case is of interest in this connection.

In Experiment 8 (May 22, 1905), two hours and forty-seven minutes after release following an occlusion of 60 minutes, abdominal movements began. Twenty-four minutes afterwards, on stopping artificial respiration, these movements spread upward to the thorax, producing fair ventilation of the lungs for three minutes, after which artificial respiration had to be started again as signs of asphyxia (stiffening of the forelimbs in extensor spasm) came on. The same series of events was repeated 46 minutes later. True bulbar respiration never returned.

This phenomenon has been observed so frequently that we may say, almost as a general rule, that in those cats which do not recover the power of normal permanent breathing of the bulbar type, imperfect respiratory movements seem to start from the lower part of the cord involving the hind legs and abdomen and then, if sufficiently strong, extending up to the thorax.

The Resuscitation of the Bulbar Vasomotor Mechanism.—We

[14] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 339.

have given in another place[15] the physical requirements, in the matter of a suitable blood pressure, for the continued activity of the heart. Still more striking examples are given by Goltz.[16] In dogs whose spinal cord had been transected in the lower dorsal region and allowed to recover, destruction of the cord below the level of the transection was followed by death in thirty hours or less. According to Goltz the cause of death was the low blood pressure.

It is clear, from a consideration of these facts, that a certain blood pressure is necessary for the continued action of the heart and respiratory mechanism. So long as the peripheral vessels remain widely dilated, and the blood passes through with relatively little resistance, this condition will not be fulfilled. The arterioles must be constricted to their normal diameter, the splanchnic vessels must give up their enormous volume of stagnant blood, and the natural resistance to the blood flow be reëstablished. All this is a part of the work of the vasomotor mechanism. Before an animal can be considered as resuscitated, we must, therefore, restore the system or center which maintains the necessary blood pressure. In selecting a method for starting the heart, we must keep in mind that it is necessary to maintain, or very soon to establish, a circulation through the respiratory and vasomotor centers, particularly through the latter. If a blood pressure sufficient for the activity of the heart can be maintained, the oxygenation of the blood can easily be accomplished. The maintenance of a sufficient blood pressure by any means which is not in itself injurious, or which does not curtail the flow of blood through the vasomotor and respiratory centers is, however, far from easy of accomplishment. Aside from the resuscitation of the cortical centers, to be discussed in a subsequent section, we may regard the restoration of the vasomotor center as the completion of the final link in the vital chain.

The Relative Resistances of the Vasomotor and Respiratory Centers to Cerebral Anæmia.—The exact moment at which the vasomotor center ceases to function is not so easy to determine as the time at which the respiratory center fails, as there is no such

[15] Pike, Guthrie and Stewart, *Jour. of Exper. Med.*, 1908, x, 371.
[16] Goltz, *Archiv für die gesammte Physiologie*, 1873, viii, 460.

sudden cessation of a very noticeable phenomenon connected with the former as with the latter mechanism. Instead of a last great movement, the vasomotor center fails gradually, and the time of cessation of function is further masked by the fact that there are accessory vasomotor centers in some animals which assume a part of the function of the medullary center on the failure of the latter. It is our opinion that the vasomotor center is more resistant to anæmia than the respiratory center, and attains a certain degree of functional activity after the restoration of the cerebral circulation before the respiratory center gives any sign of its activity. In this condition, we must remember that a part of the vasomotor mechanism is not subjected to anæmia, while no accessory respiratory centers concerned in the first movements in the resuscitation period exist outside the medulla oblongata. We are therefore restoring only a part of a system in the first case and a whole system in the second. In order to make conditions strictly comparable, we must destroy the function of all vasomotor centers as well. This is accomplished in asphyxia or any other form of general death. Exact observations, aside from Hayem's[17] work on death from hemorrhage, are not at hand in sufficient numbers to decide unequivocally which center persists longer in general death, but we believe that the vasomotor center is the more resistant, and that it is also somewhat easier to restore to a certain degree of functional activity than the former center. In cerebral anæmia, with all the systemic vessels, aside from those of the head open, Traube-Hering curves have sometimes appeared in the blood pressure tracing after total cessation of respiratory movements. In these experiments, it must be assumed that the vasomotor center is the more tenacious of life.

We have previously discussed the condition of the vasomotor centers in the spinal cord, and will quote only one new experiment here.

The pads on the hind foot of a cat, when placed in contact with ice, became very red at a time in the resuscitation period when no eye reflexes had returned and when the bulbar vasomotor center had, in all probability, not recovered its tone. The reaction was brought about, therefore, either through the vasomotors of the cord, or through a local mechanism.

[17] Hayem, *Arch. de physiol. norm. et path.*, 1888, Ser. 5, i, 103.

Although the spinal cord centers, or what we have supposed to be spinal vasomotor centers, maintain a fair blood pressure in the absence of the cerebral centers, unequivocal reflexes are not easy to obtain through the former, at least in the occlusion period and early in resuscitation. There seems to be a marked automatic element in their activity, but further work on this point is necessary. It is possible that this absence of spinal vasomotor reflexes, as we will point out later, may be due to spinal shock.

The tonicity of the bulbar vasomotor mechanism begins to return, in the resuscitation period, as a rule before the respiratory center begins to discharge, and, as is the case with the respiratory center, there is often a period during which the blood pressure may be rising, but when reflex changes in pressure do not occur through stimulation of the vagus or brachial nerves. Similarly, a time may often be found in the occlusion period when the blood pressure is still high, and the bulbar vasomotor mechanism undoubtedly functional, when stimulation of the afferent nerves produces no effect on the blood-pressure curve. We have attributed the persistence of pressure after the cessation of the reflex effects, and this early rise in pressure before the opening up of the reflex pathways to the automaticity of the nervous mechanism involved.

TABLE I.

Showing the length of the occlusion period and the time of return of deglutition, as compared with the return of respiration.

Date.	Length of Occlusion.	Time after Release of First Gasp.	Time after Release of First Swallowing Movement.
March 3, '05	6 minutes	40 seconds	3½ minutes
March 18, '05	12 min. 45 sec. Heart stopped 15 to 20 min. Started by massage.	Less than 38 min.	52 minutes
March 29, '05	10 minutes	5 min. 40 sec.	14½ minutes. Several movements.
March 29, '05	20 minutes	5 minutes	27 minutes
June 7, '05	31 minutes	9 minutes	6 hours

Considered either from the view point of resistance during the occlusion period, or the return of function during the resuscitation period, the respiratory and vasomotor mechanisms show a greater vitality than either the extrinsic cardiac or the swallowing mechanisms.

The Resuscitation of the Swallowing Mechanism.—The resuscitation of this mechanism is of peculiar interest from the fact that swallowing is so clearly a reflex act. The time in the resuscitation period at which the first swallowing movement was observed is given in Table I. In all experiments the respiration returned a considerable time before swallowing was observed. In all experiments, too, the blood pressure had risen to a considerable height. Swallowing has, however, been observed before the return of the eye, ear or fore-limb reflexes. Experiment 4 may be cited as a case in point. After long occlusions, swallowing has been observed to return along with the rolling movements of the eyes and the secretion of tears a considerable time after the restoration of the cerebral circulation, *e. g.*, in one experiment, six hours after an occlusion of thirty-one minutes.

When the order of disappearance of the reflexes and functions of the bulbar centers is studied, it will be noted that swallowing may persist longer than the corneal or light reflexes, but ceases before the end of the secondary series of respiratory gasps or the failure of the vasomotor center. The time relations of the cessation of some of these functions are shown in the following protocol.

Experiment 9.—(April 19, 1905.) Cat. Ether. Tube in larynx.

3:12 P. M. Occluded head arteries.

3:12:15. Corneal reflex gone. Respiration stopped, pupils dilated, legs straightened out. Started artificial respiration.

3:12:25. Swallowed.

3:12:48. A slight respiratory movement.

3:13:05. Another slight respiratory movement.

3:13:15. Pupil about one half maximal dilation. Several strong swallowing movements.

3:13:40. Pupils now much wider, nearly maximum. A strong gasp occurred.

3:14:05. Several strong gasps in quick succession, and kicking movements of hind limbs.

3:15. Struggling of whole body, except head. Tail lashing violently for a long time.

3:15:30. Strong struggling movements. Strong lashing of tail, and scratching movements of hind limbs.

3:16:05. All movements stopped. There have been no respiratory movements for a minute.

3:18. Pulse 258 in the minute.

3:19:20. Hind limbs now quite limp. *No response on pulling or striking.*

3:22. Put thermometer into rectum. Hips twitching when thermometer was passed. Rhythmical contraction of rectal and vaginal sphincters going on. Temperature 36½° C.

3:25:30. Moving thermometer in rectum causes contraction of vaginal and rectal sphincters and whole perineum.

3:30:15. Released head arteries.

3:42. Good kicking movements of hind legs on putting thermometer into rectum. Rhythmical movements of rectal sphincter, about twelve a minute.

In this experiment, the persistence of the swallowing mechanism is intermediate between that of the eye reflexes and of the bulbar respiratory and cardiac mechanisms. The same time relations appear in many other experiments, and we have never observed the persistence of swallowing after the respiration had permanently ceased, or the blood pressure fallen very low in the occlusion period.

The activity of the swallowing center, as we have shown in another place, may be conserved for a time when other fluids are substituted for blood.

The effect of the fresh defibrinated blood, artificially circulated through the head of a dog, in maintaining the irritability of the œsophagus and the integrity of the nerve endings in the tongue, and upon the submaxillary glands and the sympathetic endings in the pupil as well, is shown in the following experiment.

Experiment 10.—(February 28, 1905.) Medium sized dog. Ether.

2:40 P. M. Drew off 830 c.c. blood. Began artificial respiration.

3:15. Tied aorta and put cannula in the central end. Heart soon stopped.

3:26. After elevating hind end and bandaging abdomen, tied inferior vena cava and put cannula in heart end. Tied subclavian arteries and veins.

3:29. Tied a ligature around heart in auriculo-ventricular groove, leaving out inferior and superior cavæ. No eye reflexes.

3:30. Started artificial circulation with a double bottle arrangement. The blood would not run out of the cannula in inferior cava, although the auricle and superior cava and inferior cava down to the cannula were full. Removed clot. Blood now runs off.

3:40. Circulation was really started.

3:48. Stimulated left vago-sympathetic, in continuity, without tying. Good bulging of the eye and dilation of pupil. Secretion, or possibly liquid gastric contents, started to flow from the nose. There were a few drops before, but it is now much increased. The pupils have narrowed considerably since the artificial circulation started.

3:50. Stimulated right vago-sympathetic, in continuity, without tying. Marked bulging of the eye, opening of palpebral fissure, and dilation of pupil.

3:59. Still considerable liquid dropping from nose. Good bulging of eyes on stimulation of the vago-sympathetics.

4:02. Opening of left eye and dilation of the pupil on stimulation of vago-sympathetic. Immediately after stimulation much liquid runs out of mouth and nose, evidently from the œsophagus, and contains small granules of food.

Either the œsophagus contained liquid, which stimulation of the vagus caused it to expel, by causing contraction, or vomiting was caused from the stomach by vagus stimulation. The mucous membrane of the mouth and tongue is moist and well flushed with blood. Vaso-dilation seems to be present.

4:23. Marked dilation of pupil and bulging of right eye on stimulation of right vago-sympathetic. Exposed left chorda tympani and Wharton's duct. The duct is empty. Stimulation of chorda does not cause filling of duct.

4:35. Stimulated each vago-sympathetic. Marked opening of eyes but now little or no dilation of pupil. Stimulated central end of left lingual nerve. No reflex movements.

4:43. Stimulation of left vago-sympathetic causes marked bulging of eye, some retraction of nictitating membrane and slight dilation of pupil.

4:46. Stimulation of left hypoglossal nerve causes strong contraction of tongue. Motor endings intact.

4:53. Trephined and stimulated motor area on right side. No effects of any kind. No eye movements nor change in pupil on left side. No movements of neck nor left forelimb. The jaws are now showing some rigor (decerebrate rigidity?). Stimulated left vago-sympathetic. Fair dilation of pupil.

4:55. Again stimulated right motor cortex. No effect even with strong stimulation. Cut out gray matter and stimulated corona radiata. No effect. Stimulated axillary nerves on left side. Good contraction of muscles. Direct stimulation also caused good contraction. Stimulated œsophagus. Good contraction. The œsophagus seems to retain its excitability better than anything else in the neck. Stimulated left sciatic nerve. No effect either direct or reflex. Stimulated hind leg muscles directly; no effect.

On post mortem, found air bubbles in vessels at base of brain, *e. g.*, basilar, and also on cortex.

All through this experiment from the time the artificial circulation was properly started, the flow of blood was good and the carotids showed a good pressure. The blood was in an air-pressure bottle under about 100 mm. Hg.

It will be noted that the peripheral nerves and muscles, and particularly the œsophagus, retained their irritability for a considerable time after the motor cortex became inexcitable. The motor endings in the tongue were also fairly well conserved by the artificial circulation.

The œsophagus remains irritable, both to direct stimulation and to stimulation of the vago-sympathetic, for long periods of time under the influence of artificial circulating fluids. Stimulation of the vago-sympathetic may also affect the œsophagus at a time when there is no effect on the eye, although the eyelid responds readily to direct stimulation. This difference in the reaction of the two structures may be due to the interpolation of a synapse on the path to the eye, while there is, so far as we are aware, no synapse on

the path to the œsophagus. Instances of such phenomena have been given elsewhere. In one experiment, Number 11 (March 1, 1905), the œsophagus about two inches above the heart was observed to beat with almost the same rhythm as the auricles. We do not know the cause of these rhythmical contractions, but offer the suggestion that they might have been due to the action current of the heart. As is well known, the action current of the heart may, under certain conditions, excite the phrenic nerve and cause rhythmical contractions of the diaphragm which are synchronous with the heart beats.[18]

The Comparative Argument for the Automaticity of the Respiratory Mechanism.—Additional evidence of the automatic or reflex nature of the origin of cardio-inhibitory and vasomotor tone and of the respiratory movements, as we mentioned above, may be obtained from a comparative study of these mechanisms with that for deglutition. We have pointed out elsewhere that the synapse is a weak link in the nervous reflex arc. So far as we can see, there is no apparent reason why two such arcs as that through the respiratory center and that through the swallowing center should not be resuscitated at approximately the same time. As Marckwald[19] has shown, there is a very intimate physiological, and probably anatomical, connection between these two centers. If respiration were reflex, as swallowing so evidently is, we should expect a very close correspondence in the time of resuscitation of these two mechanisms. But when we look for such correspondence in time, we do not find it. On the contrary, as will appear from the various protocols quoted and from Table II, we find that respiration returns considerably earlier in the resuscitation than swallowing does. The most obvious conclusion is that respiration occurs in the absence of afferent impulses. The time at which stimulation of the afferent nerves begins to affect respiration corresponds much more closely with the time at which swallowing first occurs.

The vasomotor and swallowing mechanisms may be compared in a similar way, and again we conclude, from the early appearance of vasomotor tone, and the relatively late return of the influence of

[18] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 339.

[19] Marckwald, *Zeit. f. Biol.*, 1888, xxv, 1.

afferent nerves upon the blood pressure, that there is a very marked automatic element in the origin of the vasomotor tone. Very probably the circulation of the blood to the different organs according to their needs is brought about reflexly, but it seems to us that the height of the general systemic blood pressure may well be due to an automatic mechanism.

When we compare the time of the resuscitation of the cardiac centers with that of the swallowing centers, the results, and therefore the conclusions also, are somewhat different. The respiration becomes established, and the blood pressure reaches a considerable height—perhaps its maximum, before reflex cardiac effects appear. Then, suddenly, the reflex arc is again integrated and the heart becomes visibly affected by afferent impulses to the bulbar centers. In point of time, cardio-inhibition appears much nearer the return of swallowing than do the other two functions. We conclude, therefore, that cardio-inhibition and acceleration are very largely reflex in origin.

When we study the relative order of failure of these four functions during the occlusion period, we find further evidence in favor of these conclusions. The march of events is so rapid, and certain other conditions, to be mentioned presently, which enter in here, complicate things so much that this evidence is probably not so trustworthy as that drawn from observations during the period of resuscitation. The swallowing movements fail first, then the cardiac impulses and finally the respiration and vasomotor tone. We might reasonably expect therefore that, since the synapse is the weak link, the reflexes would fail first, and the automatic functions persist longer.

The only disturbing factor is that entering into the deportment of the heart, since cardiac acceleration persists longer than we would expect a pure reflex to do. It is possible, but perhaps not probable, that the mere asphyxiation of the cells of origin of the inhibitory nerves serves to excite them until they succumb, at which time the accelerators become active, as we have previously pointed out.[20] It is, however, noteworthy that even the cardio-acceleration fails before respiration, and it has not been shown that the cardiac

[20] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 349.

centers ordinarily remain active when afferent impulses are excluded, without, at the same time, interfering with the afferent paths.

Bearing in mind all of the phenomena of occlusion and resuscitation, we conclude that respiration and vasomotor tone are, at their inception in the resuscitation period, almost or quite automatic; later, afferent impulses begin to affect them; the cardiac centers, on the contrary, are, under normal conditions, almost or quite dependent upon afferent impulses for their activity.

III. THE RESUSCITATION OF THE HIGHER NERVOUS MECHANISMS.

The Return of the Eye Reflexes.—The relative time of the failure of the eye reflexes, as compared with the respiration, during the occlusion period, and of their return in the resuscitation period have been given in detail in another paper, and will not be dwelt upon at length here. There are, however, certain new facts in the action of the cervical sympathetic upon the pupil that we wish to present in this paper.

The artificial circulation of fluids through the head does not, as a rule, long maintain the integrity of the sympathetic endings about the pupil. This is shown in the various protocols already cited in the first of these studies. Again, the cervical sympathetic, when stimulated, may fail to affect the pupil, but the lachrymal gland will secrete, apparently in response to the stimulation.

In one experiment (12) in which the head arteries were occluded for several short periods, and a mixture of fresh defibrinated blood and Locke's solution circulated through the head later, stimulation of the left vago-sympathetic nerve, in continuity, caused enormous dilation of the left pupil, with bulging of the eye and retraction of the nictitating membrane. At the end of one minute, the left pupil was still widely dilated, and the right pupil was also dilated, although not as much as the left. Twenty-five minutes later, stimulation of the left vago-sympathetic gave the same effect on the left eye as before. After an interval of a few seconds, the right pupil also enlarged, the lateral half dilating much more than the medial half. Two minutes later, the left vago-sympathetic was again stimulated. Some dilation occurred in the left pupil, which had remained much dilated since the previous stimulation, but there was no effect on the right pupil, which had remained the same size. Very shortly afterward—three or four minutes—both pupils became totally insensible to stimulation of the cervical sympathetic.

From the protocols given in the first of these studies, we see that the œsophagus may respond to stimulation of the vago-sympathetic trunk in continuity after the pupil fails to respond, although the pupil may still respond to direct stimulation in some instances.

In Experiment 13 (April 3, 1905) the left pupil remained much narrower than the right for a long time in the resuscitation period. Three and one fourth hours after release, following an occlusion of 50 minutes, neither section nor stimulation of the left vago-sympathetic caused any change in the left pupil, which remained contracted. The right pupil was at maximal dilation.

We have supposed there might be a synapse on the sympathetic pathway to the eye, which broke down, under the adverse influences of anæmia and the artificially circulated fluids, sooner than a direct pathway free from synapses.

We have observed, also, that, when the eyes are widely opened and bulging, and the cornea hard, probably indicating a high intra-ocular pressure, the stimulation of the cervical sympathetic may not cause maximum dilation of the pupil, and that whatever dilation occurs disappears soon after the cessation of stimulation, the pupil quickly returning to normal.

There has been much discussion among authors as to whether the respiratory or the pupillo-motor center is the more resistant to cerebral anæmia, and which was the first to be resuscitated. Hayem[21] states definitely that the respiratory center is the last of the cerebral centers to fail in death from hemorrhage. We have found that the respiration returns in cats before the pupils are contracted. Herlitzka,[22] in discussing some of D'Halluin's[23] criteria of resuscitation, states that contraction of the pupil may be a purely hydraulic phenomenon[24] depending upon pressure factors alone, and is not, therefore, an accurate index of the functional activity of the pupillo-motor center. In the absence of all other cerebral functions the contraction or dilation of the pupil may not be a trustworthy sign of functional activity, but if other centers are active, we see no reason why the state of contraction of the pupils should

[21] Hayem, *loc. cit.*
[22] Herlitzka, *Arch. ital. de biol.*, 1905, xliv, 93.
[23] D'Halluin, *loc. cit.*
[24] Mosso, cited by Herlitzka, *loc. cit.*

not be regarded as a valid index of the functional activity of the pupillo-motor center. Indeed, we have experimental evidence that the state of contraction of the pupil does not always depend upon blood pressure alone. We have pointed out, in our first paper, the difficulty of distinguishing between the effects due to the cerebral center and those due to sympathetic influences and will not enlarge upon them here.

The mere contraction of the pupil, as it occurs in the return of cerebral function, must be carefully distinguished from the contraction and relaxation due to changes in the intensity of the light. The pupils may be contracted in the resuscitation period, and the light reflex absent. During the succeeding days the pupils may be wide, especially during the spasms, and the light reflex present, in some degree at least.

The widening of the pupils during spasms, as we have stated elsewhere, is an almost, or even quite, constant occurrence. The dilation may not appear as soon as the spasms begin, but some hours after, and is always plainly evident in those animals which are kept alive on the day following the operation.

In Experiment 14 (May 13, 1905), for example, in which the head arteries were occluded for one hour, permanent extensor spasms of the forelimbs were noticed five hours and 46 minutes after release of the head vessels. Fairly strong spasms of the forelimbs began six hours after release, and increased in severity as time elapsed. It was not until eight hours and 20 minutes after release that the pupils were observed to dilate during the spasms, which, at that time, were of considerable severity.

We have mentioned in another place the fact that the cornea becomes sunken and furrowed during the period of anæmia, if the occlusion is at all prolonged, and that the cornea may again become smooth and firm in the resuscitation period. The time in minutes after occlusion at which the corneal tension begins to decrease, and the time in minutes after release when the cornea again becomes firm, are shown in Table II, which gives also the length of the

TABLE II.

Length of Period of Occlusion in Minutes.	Minutes after Occlusion when Tension Begins to Lessen.	Minutes after Restoration of Circulation when Tension Returns.
31	3 min. 50 sec.	21 min. 30 sec.
10	7 min. cornea furrowed	18 min. 30 sec.
15	———	32 min.
8	30 sec.	5 min. 40 sec.

occlusion period. The effect of intraocular tension upon the response of the pupil to stimulation of the cervical sympathetic has already been mentioned.

The intra-ocular tension may be maintained for a time, or even restored in rare instances, by a good circulation of fresh defibrinated blood through the head end of an animal.

The Temperature of the Animals in the Post-Anæmic Period.—The question arises whether the temperature of the animal remains the same or whether the heat regulating mechanism, sharing the general derangement and sometimes apparent hyperactivity of the other nervous mechanisms, maintains an abnormally high temperature for a time.

At the conclusion of the operation, and after the respiration has returned so that the animal is able to breathe spontaneously, the temperature, taken in the rectum, is often subnormal. The temperature as measured at varying intervals during the post-anæmic period is given in Table III.

The temperature immediately before occlusion of the cerebral arteries was sometimes subnormal, *e. g.*, 36° C., particularly if the dissection of the vessels had been tedious, but was always markedly below normal at the end of occlusion, falling to 32° C. in one instance. A fall of 0.6° C. occurred during an occlusion of ten minutes. In the cases where marked cerebral injury resulted from the occlusion, the temperature often fell one or two degrees after the release of the cerebral arteries, but subsequently rose again. In a case of severe convulsions the temperature was 39.7° C. on the day after the operation. In another case where no violent convulsions were observed after the first few hours, the temperature was 32° on the day after the operation. Another animal which recovered without abnormal symptoms had a temperature of 38.4° C. on the second day. In some instances where the temperature fell very low, the animal was put in a covered box which could be heated by a burner beneath it. Sometimes a rise of temperature followed this procedure, the rise continuing after extinguishing the flame or removing the animal from the box, and reaching a point somewhat above normal. Putting in the ice chest, primarily for the purpose of controlling the spasms, caused a fall of temperature,

TABLE III.

Showing the length of the occlusion period, and the temperature of the animals at varying periods after the release of the cerebral arteries.

Number of Experiment.	Length of Occlusion.	Temperature (Rectal).	Time after Release.
I.	60 minutes 30 seconds	32° C.	4 min.
		31	24 "
		30.5	37 "
		30.4	47 "
		30	1 hr.
	Artificial heat	29.8	1 " 27 "
		29.5	1 " 42 "
		30	2 "
		30.30	2 " 8 "
		31.2	2 " 19 "
		32.8	2 " 43 "
	No more heat	33.5	2 " 58 "
		33.8	3 " 33 "
	Artificial heat	33.5	5 " 45 "
		33.5	5 " 58 "
	Burner turned out	33.5	6 " 4 "
		34.2	6 " 58 "
		34.4	7 " 6 "
		Put in ice chest	
		32	7 " 33 "
	Not on ice	32	7 " 47 "
		32.2	8 " 5 "
	In ice chest		
		30.4	8 " 38 "
		30.4	8 " 56 "
		30.4	9 " 8 "
		30.7	9 " 33 "
		32.4	12 " 23 "
		Death	15 " 8 "
II.	26 minutes		
		33.3	5 "
		32	6 "
		33.5	21 "
		33	34 "
		31	4 " 37 "
		32	20 " 50 "
		32.9	26 " 20 "
III.	10 minutes 7 seconds	35.4	During occlusion
		35.2	5 "
		35	14 "
		34	1 " 24 "
		39.7	21 " 24 "
IV.			
		34	30 "
		37	20 "
V.	45 minutes		
		36.5	At release
		36	14 "
		35.3	26 "
		34.8	38 "
		34.6	51 "
		34.3	1 " 5 "
		33.4	1 " 53 "
		33.3	2 " 54 "

the fall sometimes continuing after the removal from the ice. In general, the temperature regulating mechanism of the animal suffers along with the other cerebral mechanisms, the extent of the variations of the temperature depending, in a somewhat general way, upon the severity of the general effects of anæmia. If the animal recovers its other cerebral functions the temperature regulation, as a rule, again becomes normal.

Von Bechterew[25] and others were led, from a consideration of clinical evidence, to suspect the existence of a cortical center for the secretion of sweat. In a recent paper, von Bechterew[26] gives the experimental evidence for the existence of such a center. On the basis of this work we may explain the cause of the sweating in Green's[27] case by supposing that the center for the secretion of sweat, like many other cortical centers, is not acting in a normal manner, or may even be hyperexcitable, as the cortical motor centers apparently are. The conditions in cats are not so easy of observation, and we have no constant results on this phase of the subject. In most instances, in which attention was directed to this point, the pads of the feet were moist.

Some glycosuria is always present in animals which live more than a few hours. This is not due to the anæsthetic alone, as was shown by control experiments. It is possible that the injury or excitation of the medullary "diabetic center," similar to that which produces puncture glycosuria, may explain the appearance of dextrose in the urine. The experiments of von Bechterew showed that stimulation of a particular area of the motor cortex in the dog caused an increased flow of urine, and, frequently also, glycosuria. The derangement of such a cortical mechanism in the cat would explain the glycosuria so constantly noted.

The Resuscitation of the Cerebral Motor Cortex.—The activity of the motor cortex is not well maintained by artificial circulation of defibrinated blood or other fluids, as is shown by the protocols of the experiments already quoted. Moreover, the white matter, *e. g.*, the fibers of the corona radiata, soon loses its excitability

[25] v. Bechterew, *Arch. f. Physiol.*, 1905, 297.
[26] v. Bechterew, *loc. cit.*
[27] Green, *loc. cit.*

under the influence of the artificial circulatory fluids, as shown in Experiment 10. The cortex is inexcitable during the period of anæmia, and may fail to recover after the normal circulation is reëstablished.

In Experiment 15 (March 4, 1905) the head arteries of an adult cat were occluded for ten minutes. The pupils slowly contracted after release of the head vessels, but began to dilate again about one hour after release. No corneal or other reflex ever returned. Shortly after the two pupils began to dilate (62 minutes after release) about 100 c.c. of a mixture of fresh defibrinated blood and Locke's solution (45 c.c. blood diluted to 275 c.c.) was injected into the aorta under a pressure of 100 mm. of mercury to increase the volume of circulating fluid. The pupils became narrow in nine minutes after the injection was begun. Three minutes later, stimulation of the left vago-sympathetic produced dilation of the left pupil, retraction of the nictitating membrane and bulging of the eye. The dilation of the pupil disappeared almost as soon as the stimulation was stopped, but the bulging of the eye persisted a little time. Eighteen minutes after starting the injection, gasping movements occurred, increased in strength and persisted thirteen minutes. At the end of this interval, 31 minutes after starting the injection, the left pupil was dilating and the blood pressure in the carotid was low. About 30 c.c. of the blood mixture was now injected, and respiratory movements came back almost at once, but grew feebler in four minutes. Marked spasms of the face muscles occurred. Thirty-six minutes after starting the first injection a third injection of the blood mixture was begun. Artificial respiration was suspended for 40 seconds, and then started again. The respiratory gasps began almost immediately afterward. At this time, 41 minutes after the first injection, the animal puckered up its face and closed its eyes tightly. The pupils dilated greatly, and both respiratory gasps and facial spasms ceased in one and one half minutes. A fourth injection was begun 65 minutes after the first. The pupils again contracted somewhat, only to dilate again, and nasal and lachrymal secretion appeared. A fifth injection occurred 72 minutes after the first, and again the pupils contracted. At this time, stimulation of the left vago-sympathetic caused dilation of the pupil. Eighty-one minutes from the beginning of the first injection, the skull was trephined and the left motor center stimulated. There were no movements of any kind. The cortex was then excised and the corona radiata stimulated, with the same result as for the cortex. Seven minutes later, the experiment was stopped. There was some twitching of the right eyebrow at this time, and the heart was still in good condition.

In Experiment 16, a part of the protocol of which is here cited, the motor cortex was excitable to direct stimulation fifty minutes after starting the heart.

Experiment 16 (March 18, 1905). Cat. Ether. Tube in larynx, artificial respiration.

11:28 A. M. Occluded head arteries.

11:40:45. Released head arteries. Soon after this, animal stopped breathing. Opened thorax and massaged heart directly.

12:05 P. M. Heart started again.

12:57. Pupils well contracted. No light reflex but corneal reflex is present.

12:57:30. Light reflex back. Swallowing. Marked lachrymal secretion.

1:02. Pupils of normal size. No reaction of animal to loud sounds produced by clapping hands. Shoulders and head move when board is sharply struck.

1:09. Touching interior of ear causes movements of eyelid on same side. These are produced especially well by flicking the ear with the finger. No movements of eyelids on opposite side.

1:22. Moved head on whistling in ear but not on blowing into it, though blowing caused lid movements.

1:45. Put ammonia in the nostrils. No effect. Put ammonia in the mouth. Strong movement of head and neck and some movements of forelimbs. Trephined skull.

1:55. Stimulated motor cortex. Got jaw movements of opposite sides. No other movements except perhaps slight movement of opposite forelimb. As soon as the skull was opened, occasioning some hemorrhage, deep regular spontaneous respiratory movements began, involving head, neck and chest down to diaphragm. It was not observed whether the diaphragm took part. These movements were much more effective for respiration than the previous gasping respiratory movements.

Similar results have been obtained by Scheven[28] on rabbits. This observer found that the white matter of the central nervous system was more resistant to anæmia than the gray matter. Stefani and Cavazzani[29] found that the rabbits' peripheral nerves would retain their conductivity for afferent impulses up to ten minutes after being deprived of their blood supply.

We have previously pointed out[30] that the convulsions occurring during the resuscitation period after cerebral anæmia[31] are not necessarily due to the motor cortex, since the same phenomena occur in animals in which the brain has been divided at about the level of the roots of origin of the fifth nerve.

Micturition sometimes occurs during these convulsions, with the anatomical connections intact. While it is possible that the expulsion of the vesical contents is due to the spasm of the abdominal muscles, there is also the possibility of involvement of the cortical motor center for the bladder.

[28] Scheven, *Arch. f. Psychiatrie*, 1904, xxxviii, 926; *ibid.*, 1904, xxxix, 169.

[29] Stefani and Cavazzani, *Mem. letta all' academia medico-chirurg. di Ferrara*, 1888, cited by Scheven.

[30] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xx, 72.

[31] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 307.

Mewing, indicating recovery of the cortical area for phonation, has been observed twenty-two and one half minutes after release from an occlusion of five minutes. The light reflex was well marked at this time, and the pupils were of normal size. The spasms were marked. The mournful mewing was evidence of a considerable degree of cortical activity. In another experiment, of fifteen minutes occlusion, the spasms were of marked severity six hours after release. The cat uttered a peculiar "yowl" from time to time during the spasms, and particularly during the latter stage of a spasm. This cry may have been from the corpora quadrigemina,[32] and not from the cortex. The cat was found dead fourteen hours later.

Consciousness, in some degree, has been observed twenty-five minutes after release from an occlusion of nine and five sixths minutes. There was no light reflex present at this time, but the tail was wagging. Flicking the fore paws caused strong gasping movements. Consciousness, and the reflexes have persisted throughout an imperfect occlusion of eighteen and one half minutes, followed, thirty-five minutes later, by a second imperfect occlusion of thirty minutes. The fore limbs, particularly the left, were weak until the animal's death eight days later, and straddled out from the body in walking. A spastic gait is often observed when the animals begin to walk about after recovery from the operation.

Voluntary movements of the head, neck, shoulders and fore limbs, but no corneal reflex, and respiratory movements involving the muscles down to the shoulders, have been observed eight minutes after release from an occlusion of six minutes. Voluntary change of position was observed in another animal three and one half hours after release from an occlusion of thirty-one minutes. In another case, no voluntary movements ever returned, even twelve hours after release from an occlusion of forty-five minutes, although the circulation to the head was good all of the time.

Continuous sleep, with tightly closed eyes, was observed twenty hours after release from an occlusion of five and five sixths minutes following an incomplete occlusion of nine and one half minutes.

[32] Ferrier, Functions of the Brain, 2d edition, New York, 1886, p. 166.

The rectal temperature was 38.7° C. The clonic spasms were entirely gone, and did not appear on stimulation. The respiration was thirty-three a minute, and regular. The light and corneal reflexes were well marked. The animal swallowed salt solution placed in the mouth, and tickling the ear caused the ordinary movements of it.

The Senses after Recovery from Anæmia.—Sight is often apparently normal after cerebral anæmia, but apparent total blindness has also been met with. Blindness did not occur immediately, but developed within a week or ten days after occlusion. Strangely enough, the light reflex appeared to be present in some degree. The corneal reflex still remained, for the eyelids were closed when the eyeball was touched. The pupils were often wide and staring, but were sometimes seen to be narrowed in bright light. The location of the lesion, whether in the retina or in the cerebral center, and its nature are unknown.

We have observed wide and staring pupils in animals whose sense of sight was still present. The nature of the impression made on the disordered brain is a matter of conjecture. The animals howled when anyone came into view, but showed neither fear nor affection. The vision often becomes apparently normal so far as we were able to determine by our tests. In one experiment, the sense of sight had evidently returned three hours and fifty minutes after release from an occlusion of nine minutes and fifty seconds.

Hearing.—Like sight, the auditory sensation shows varying degrees of recovery. After anæmia and during the period of convulsions, there is often apparently increased sensitiveness to slight sounds. The ticking of a watch is often sufficient to cause convulsions, all other stimuli being excluded. In favorable cases, the return of hearing is apparently complete. Total deafness, as a result of cerebral anæmia is rather doubtful, but deafness to ordinary sounds is well established, for example, calling, and the mewing of kittens were unnoticed. A loud, sharp report near the ear usually attracted some attention. The location and nature of the lesion are unknown.

Hearing returns somewhat earlier in the resuscitation period

than sight. In one experiment, in which the heart was started by massage after a stoppage of fifteen to twenty minutes, loud sounds caused no reaction one hour after the heart began to beat. One hour and twenty minutes after starting the heart, the head moved on whistling in the ear, but not on blowing into it, although this blowing caused eye movements. Again in another experiment in which the heart stopped for about ten minutes after an occlusion of six minutes, the cat moved on striking the board sharply fifty minutes after starting the heart, but not on calling or clapping the hands. Hearing had probably not returned in this last experiment.

The recognition of sounds returns somewhat later than the mere audition. In the last experiment in the preceding section (in which there was an occlusion of nine and five sixths minutes), the animal recognized sounds, *e. g.*, "pussy," three hours and twenty minutes after the release of the head arteries. Thirty minutes later there was evidence of sight. In another experiment, the sense of hearing was apparently abnormally acute fifty minutes after release after an occlusion of three and one third minutes, the animal starting at any sound, and going into tonic extensor spasms. Two hours later, the cat was sitting in the normal position of a couchant cat, but with the head turned sharply to the right. A loud sound caused it to change its position, the animal lying on its belly with its head straight in front.

The results on *taste* and *smell* are not so definite. Ordinarily the animals eat well, and manifest a preference for certain kinds of food. Food may be taken when the animal is blind and deaf. Milk held near the nose may not be lapped unless the nose is actually touched with it, but, once tasted, is eagerly eaten.

Touch.—After the exaggerated sensibility to touch of the first few hours or days following anæmia, the sense of touch becomes apparently normal in animals which show otherwise favorable symptoms. There is the normal response to stroking the fur. Even a blind and deaf cat showed this response and would often mew when stroked. We believe it is a response to touch as a sensation rather than the response of a spinal animal to a stimulus. The reflex response to tickling the ears was always observed if a

sufficient time was allowed to elapse after anæmia. Pain and temperature sensations were present in the cats which recovered well otherwise, but the responses to these stimuli could not always be interpreted in animals which suffered apparent loss of some of the other senses.

The Return of the Mental Processes.—This is perhaps the most interesting of all the phases of the resuscitation of the brain, and a phase which can be studied in detail by the clinical observer alone. Animals may be watched in the laboratory, and may manifest all the functions normal to the ordinary animal, but we are unable to tell whether the mental processes are completely restored.

So far as we are able to judge from the deportment of the animals, all the mental faculties may return after short periods of occlusion (eight minutes). The animals observed showed no noticeable change in deportment. The responses to calling and stroking were normal. A white rat allowed to run in front of one cat was at once pounced upon, and was rescued only with difficulty. On the application of a non-irritating antiseptic solution to the skin lesions which developed later, the cat showed the usual aversion to water. The general results in this case were decidedly encouraging. We have other examples of apparently complete return of the mental processes after occlusions of six minutes, seven and one half minutes, nine and five sixths minutes and sixteen and one half minutes. In this last experiment, however, we doubt the totality of the anæmia produced.

Such a favorable result does not always follow. After an occlusion of ten minutes, we have seen apparently the total loss of intelligence. We have also observed an apparent insanity along with the return of the bodily functions.

In a previous paper we pointed out that strychnine, either had no noticeable effect on the previously anæmic cord or else paralyzed it, when injected in very small quantities, before affecting the normal cord. In looking over our experiments, we find that only one cat survived the subcutaneous injection of strychnine, the rest all dying within a few hours at most. The action of strychnine in excess is to paralyze the normal cord. Since the previously anæmic area may reasonably be supposed to have a lowered vitality

and a diminished resistance to external influences, it is possible, and, in the light of our experiments, very probable that the therapeutic dose of strychnine may have an injurious effect instead of a beneficial one.

The Pilo-motor Mechanism.—Although it is probably not to be considered as exclusively under the control of the higher centers, the pilo-motor mechanism may be discussed here. Movements of the hair of the cheeks, and particularly of the vibrissæ, are not infrequent in the resuscitation period.

For example, in Experiment 17 (May 27, 1905), 45 minutes after release, following an occlusion of 45 minutes, there was constant twitching of the hair of the throat and cheeks, and of the vibrissæ. At this time the respiratory gasps were occurring at intervals of 18 seconds, and there were clonic movements of the forelimbs, with a response to striking or pinching the limb on the same side stimulated. The forelimb reflexes did not cross.

In Experiment 18, nearly five hours from the release of an occlusion of 51 minutes, the hairs on the tail were erected one minute after stopping artificial respiration (natural respiration had not returned). A second short period of asphyxia three minutes later was followed by the same result. Erection of the hairs on the tail and back has been observed during the spasms. In Experiment 14, the hairs of the tail and back were erected continuously (indicating tonic pilo-motor spasms) eight hours and 40 minutes after release from an occlusion of 60 minutes. At this time the pupils were beginning to dilate during the spasms.

CONCLUSIONS.

The experiments on cerebral anæmia have enabled us to duplicate, by an entirely different method, many of the results obtained by anatomical division or removal of parts of the central nervous system. In some respects the method of anæmia permits of greater precision than the method of division or excision, and avoids, in great measure, the disturbances due to the wound and to the hemorrhage caused by the latter method. The method of general anæmia, as Couty[33] pointed out long ago, leaves something to be desired in the matter of exact localization, but this objection may be met, in some degree at least, by appropriate methods of investigation. It is desirable that the results obtained by the method of section should be duplicated by some other method in order to eliminate as much as possible the effects due to the irritation produced by the anatomical lesion.

[33] Couty, *loc. cit.*, p. 754.

Our results show, as we believe, that, of the bulbar mechanisms studied, the respiratory is the most automatic, the vasomotor in part automatic, and the cardiac like the swallowing mechanism, almost wholly dependent upon afferent impulses for the arousal and discharge of its normal activity.

The eye reflexes return during the resuscitation period in the animals in which the cerebral anæmia has not been too prolonged. The motor cortex loses its excitability during anæmia, but may regain it after the reëstablishment of the circulation. The pilomotor mechanism is disturbed during the spasms which occur at a certain stage in the resuscitation. The temperature falls during the occlusion period, but rises again, often to far above normal, in the days following the anæmia. So many disturbing factors, such as the violent muscular contractions during spasms, enter into the problem that it is impossible to say that there is an actual disturbance of the temperature regulating mechanism although we are inclined to believe that this is the case.

All the senses return, following cerebral anæmia, but sight and hearing may afterwards fail without causing the death of the animal. The mental processes may return without any apparent deficiency, if the period of anæmia has been short. After longer occlusion, apparent insanity has been seen, and in one case, apparent total loss of mental processes occurred.

The reflex excitability of the cord returns rather early in the resuscitation period. Reflexes from the anterior part of the cord first involve muscles on the same side as the stimulus, and later cross to involve muscles of the opposite side. The spinal cord sometimes falls into much the same condition as that following spinal transection, and the scratch reflex appears. Spinal transection, when these reflexes have appeared, does not produce shock. Practically all phenomena of spinal shock may be reproduced without section of the cord. We conclude, therefore, that spinal shock is due more to the cutting off of the reflex pathways through the higher centers of the nervous system than to the stimulation of inhibitory fibers by the anæmia.[34]

[34] Pike, Guthrie and Stewart, *Amer. Jour. of Physiol.*, 1908, xxi, 371.

THE RATIO BETWEEN THE HEART-WEIGHT AND BODY-WEIGHT IN VARIOUS ANIMALS.[1]

By DON R. JOSEPH, M.D.

(From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.)

Introduction.—In this paper are given the results of observations, which I have made, on the relation between the heart-weight and the body-weight in different animals. So far as I am aware, very little, if anything, has appeared on this subject in this country, although considerable work has been published on this and closely related subjects in Europe. For that reason I have thought it worth while to collect from previous investigators a few of the figures which seemed most interesting and have arranged them in tabular form near the end of this communication.

In the literature, we find numerous tabulations which give the weight, dimensions, etc., of the various organs of the body, and also the relation between the size of individual organs and the size of the body. These have been determined for man in the normal and in several pathological conditions. Vierordt (1) gives tables showing the weights and measurements of almost every organ of the body. Bergmann (2) gives the relation of the heart-weight to the body-weight in man and several species of animals. Parrot (3) published an extensive article upon this relation in fifty or more species of birds. Hasenfeld and Romberg, (4) W. Müller (5) and Külbs (6) may be mentioned. Many of the normal ratios were determined in order to have controls for experiments upon the effects of exercise or valvular defects of the heart upon cardiac hypertrophy and related subjects. There are some variations between the figures of these investigators for an individual species, but for the most part they are fairly constant. The use of different methods may account for many of the differences.

[1] Received for publication May 6, 1908.

The Writer's Observations.—During the past five months the relation between the heart-weight and body-weight has been studied in the animals used in this laboratory for other purposes. The animals were weighed before any operation was begun, after death the heart was removed in a uniform manner (by cutting all vessels at the point where they joined the heart), the cavities were opened and carefully washed free from clots and the heart was weighed. The animals in which this relation was studied were dogs, cats, rabbits and guinea-pigs.

By the term "ratio of heart-weight to body-weight"—a term which will be used frequently in this paper—is meant the number of grams of heart tissue for every kilogram of body-weight. For example, a ratio of 4.5 would mean four and one half grams of heart tissue per kilo of body-weight.

The ages of the animals in my series of observations were unknown. No comparison, therefore, could be attempted between the ratios of younger animals and those that were older.

The animals of each species have been grouped in two ways. *First,* they were grouped according to *sex,* in order to get a comparison between the average ratio of heart-weight to body-weight in males and females of an individual species. *Second,* they were grouped according to *sex* and *body-weight.* To do this the animals of the first grouping were arranged so that the average ratio of animals having an equal (or nearly equal) body-weight could be determined. The purpose of this was to compare the ratios of lighter animals with those of heavier animals of the same species.

Grouping According to Sex.—In the following table there is an arrangement of animals according to sex only. Column six contains the "ratios."

TABLE I.

Species.	Sex.	Number of Animals.	Average Body-Weight in Grams.	Average Heart-Weight in Grams.	Average Ratio in Grams.	Average Per Cent. of Heart Weight to Body-Weight.
Dog	Male	58	8,029	59.23	7.43	0.743
	Female	60	6,038	45.46	7.61	0.761
Cat	Male	11	3,078	13.80	4.56	0.456
	Female	15	2,650	12.34	4.58	0.458
Rabbit	Male	38	1,606	4.31	2.67	0.267
	Female	66	1,697	4.57	2.70	0.270
Guinea-pig	Male	14	384	1.70	4.22	0.422
	Female	33	257	1.03	3.91	0.391

It will be seen from the table that the ratios for cats and guinea-pigs are almost the same, while that for dogs is much higher and that for rabbits much lower. As a possible explanation for the very low ratio of the rabbit, two factors may be mentioned. First, the inactivity of tame rabbits and probably a larger amount of body fat as a result. Second, the large size of the stomach and cæcum. Especially after a very full feeding this last factor would exert a marked influence upon the ratio. A ratio derived from rabbits with the weight of stomach and cæcum contents excluded would undoubtedly be nearer that found in the other animals.

The difference in the ratios of males and females in an individual species is so slight in my series as to be of small significance. In cats and rabbits the male and female ratio is almost identical. In dogs the table above shows a difference of only .18 gram of heart-tissue per kilogram of body-weight in favor of the female. In guinea-pigs there is a difference of .31 gram per kilogram in favor of the male.

In December a preliminary report upon the ratios of dogs and rabbits was made before the New York Pathological Society. In that report the tables contained half or less than half the number of animals which are included at present. It is interesting to note that after almost doubling the number of these animals, the ratios have scarcely been changed at all. This fact seems to indicate that the ratios given represent the true ratio for each species. It is illustrated in the following table, which contains the number of animals upon which report was made at the meeting in December and also the number in the present report, with the corresponding ratios.

TABLE II.

Species.	Sex.	Table Referred To.	Number of Animals.	Ratios.
Dogs.	Male.	Former report.	28	7.50
		Present "	58	7.43
	Female.	Former "	25	7.64
		Present "	60	7.61
Rabbits.	Male.	Former "	21	2.70
		Present "	38	2.67
	Female.	Former "	36	2.88
		Present "	66	2.70

From this table we see first that the addition of thirty male dogs changed the ratio only .07 grm. of heart-tissue per kilogram of body-weight; second, that the addition of thirty-five female dogs changed the ratio only .03 grm. per kilo; third, for male rabbits the addition of seventeen made a change of .03 grm.; and fourth, for female rabbits, the addition of thirty made a change of .18 grm. per kilo. This last is by far the largest variation of the four, and yet even it is, for practical purposes, negligible.

In the following table are given the highest and lowest ratios found in each sex of each species. The difference between them is quite marked in some instances, especially in the cases of male dogs and male guinea-pigs. However, there were very few animals of these two species which gave such a wide variation from the average obtained for the species.

TABLE III.

Species.	Sex.	Highest Ratio Found.	Lowest Ratio Found.	Average Ratios Given in Table I.
Dog	Male	10.55	5.35	7.43
	Female	9.51	5.72	7.61
Cat	Male	5.86	3.61	4.56
	Female	5.57	3.35	4.58
Rabbit	Male	3.42	2.07	2.67
	Female	4.47	2.00	2.70
Guinea-pig	Male	7.03	2.64	4.22
	Female	5.79	2.66	3.91

Heinz (7) (Vol. I, Part 2, page 886) gives a table of nineteen dogs with an average ratio of 9.71. Among the nineteen dogs he has seven with a ratio of 10.00 or over and eight with a ratio of 9.00 or over. In my table, with a total of 118 male and female dogs, there is only one animal which goes as high as 10.00 (and that one was a very distinct case of pathological hypertrophy), and but six animals as high as 9.00. My average for dogs, it will be remembered (Table I), was about 7.50—or 2.2 grams per kilo dog less than the average given by Heinz. That is, taking a ten kilo dog, the total heart-weight, using Heinz' average, would be 97.0 grams, while a dog of the same weight, using my average, would have a heart weighing but 75.0 grams—a difference of 22 grams. This is a much greater difference in weight than could be explained by the

use of a different method of removing the heart. It would suggest a possible difference between the dogs of Germany and of the United States, or rather of New York.

Groupings According to Sex and Weight.—In the following table the dogs have been arranged in groups according to their body-weight, those having an equal, or almost equal, weight being placed in a single group.

TABLE IV.

Body-Weight.	Female Dogs.		Male Dogs.	
	Number of Animals.	Average Ratio.	Number of Animals.	Average Ratio.
3,000– 3,999	6	8.07	1	9.69
4,000– 4,999	13	7.88	4	7.58
5,000– 5,999	12	7.90	7	6.16
6,000– 6,999	10	7.28	8	7.82
7,000– 7,999	9	7.13	13	6.99
8,000– 8,999	6	7.13	7	8.18
9,000– 9,999	3	7.48	7	7.18
10,000–10,999			2	7.23
11,000–11,999			2	7.13
12,000–12,999			4	7.22
13,000–13,999			2	6.45

We see from this table that in the case of both male and female dogs, as we pass from the lighter to the heavier ones, the ratio gradually becomes less. Or, in other words, the relation of heart-weight to body-weight follows, in general, the law of an inverse proportion. It seems to me this law might be connected with the well-known observation that the smaller the animal the higher the pulse rate. We know that skeletal muscle responds to increased activity by a hypertrophy. Since a more rapid heart beat means greater activity of cardiac muscle, may it not be that this faster rate of beat in the younger animal is the cause of a physiological hypertrophy of the heart?

When the average normal relation between the heart-weight and body-weight has once been determined, it is found that, in general, this average ratio holds fairly constant for members of that species. This is illustrated by the following instance: A dog, whose body-weight had not been previously determined, was used in the laboratory. After death, the body-weight was estimated from the weight of the heart (using an average for dogs already determined) to be

12,450 grams. To test the accuracy of this estimate, the animal was then weighed and due allowance made for loss of blood during the operation. The actual weight was found to be 12,100 grams, an error of only 350 grams. There were many more instances in which a like result was obtained by a reverse of the above illustration, *i. e.,* by estimating the heart-weight from the known body-weight, then weighing the heart to find how accurate the estimate had been. It was a very common thing to predict within one to three grams the total heart-weight.

There are, however, several factors which tend to cause individual variations from a general average ratio determined for a species. These factors may be placed under two heads. First, those which cause changes in the size of the body, such as growth (normal or abnormal), excess of fatty tissue, pregnancy, emaciation, etc.; second, those which cause changes in the size of the heart, such as increased bodily activity or pathological cardiac conditions from which compensatory hypertrophy of the heart would result.

Vierordt has shown in his published tables that for man the relative amount of heart-tissue per kilo of body-weight decreases with the advance of age, or, in other words, that the *young* individual has a higher ratio than the older individual. It is found in other tables (my own included) that the *light* individuals have a higher ratio than heavier members of the same species. It would seem that both these factors, *i. e.,* age and size, exert a certain influence upon the ratio. Of course, we usually expect a young animal to have a smaller body-weight than an older one, and if this were invariably true, it would be unnecessary to speak of age and size as separate factors. But we know that these two factors need not, necessarily, run parallel, since we have young animals of large size and older animals of small size.

Another thing which may cause a difference between the ratios of two animals of the same species, independently of a change in the size of the heart, is the presence or absence of a normal amount of fatty tissue. For instance, an emaciated dog, because of a reduction in the body-weight, would show a high heart ratio, while for an opposite reason, a very fat dog would show a low ratio. In predicting the size of the heart in a live animal, it is sometimes nec-

essary to keep this fact in mind. Aside from the three factors already mentioned, the literature contains abundant evidence that increased bodily activity or valvular defects of the heart will bring about very great differences in the ratios of an individual species. For example, Külbs took two young dogs from the same litter and kept one of them shut up in a cage, while the other was made to exercise strenuously in a treading machine. After some time they were both killed. The body-weight of each was almost exactly the same, but the heart of the exercised animal weighed 152 grams, while that of the caged animal weighed only 99 grams. This experiment gives one a definite idea of the pronounced effect which the factor of bodily exercise may have upon the size of the heart.

Whether the amount of muscular activity can account altogether for the difference in the ratios of the *different species* of animals seems somewhat doubtful, and yet this factor undoubtedly exerts a marked influence, as is shown by the following table of ratios, collected from different sources. The animals are so arranged that

TABLE V.

Species.	Sex.	Body-Weight in Grams.	Heart-Weight in Grams.	Grams of Heart Tissue, per Kilo of Body-Weight.	Author Quoted.
Tame rabbit	Unknown	1,916	4.56	2.36	Hasenfeld und Romberg
Wild rabbit	Unknown	1,120	3.07	2.76	Grober[8]
Guinea-pig	Male	384	1.70	4.22	Joseph
	Female	257	1.03	3.91	"
Pig	Unknown	49,700	225	4.52	Bergmann
Cat	Male	3,078	13.8	4.56	Joseph
	Female	2,650	12.3	4.58	"
Cattle	Male	280,000	1,450	5.35	Bergmann
	Male (cst.)	545,000	1,410	3.86	"
	Female	398,000	1,523	3.83	"
Man	Male	58,000	340	5.88	"
	Female	50,000	273	5.47	"
Sheep	Male	20,600	127	6.17	"
	Female	20.600	118	5.85	"
Horse	Male (cst.)	493,000	3,000	6.77	"
	Unknown	417,000	2,400	5.81	"
Hare	Male	3,666	28	7.70	"
	Female	3,500	25	7.17	"
Dog (American)	Male	8,029	59.23	7.43	Joseph
	Female	6,038	45.46	7.61	"
Dog (German)	Unknown	4,706	47.3	9.71	Heinz
Deer	Male	20,600	238	11.55	Bergmann
Bird (thrush)	Unknown			25.64	Parrot

the one with the lowest ratio is at the top and that with the highest ratio at the bottom of the list, the column of ratios (Column 5) exhibiting a gradual increase as one approaches the bottom. It is also very plain that the amount of bodily activity in the various animals given follows, in a general way, the same order of increase as the ratios—the more active animals being at the bottom of the table.

One or two points shown in this table are worthy of special notice. We find the tame and wild rabbits at the top with ratios of 2.36 and 2.76 respectively. Now if we look near the bottom of the list, we will find the hare, a very near relative of the rabbit, with a ratio of almost 8.0. In this case it is very easy to see the influence of activity.

The ratio of the deer (11.55) and especially of the thrush, a very active flyer and songster (25.64)—almost twelve times as great as that of the tame rabbit—are also very interesting.

It gives me pleasure to acknowledge the helpful suggestions of Dr. Meltzer given during the preparation of this paper.

BIBLIOGRAPHY.

1. Vierordt, Anatomische, physiologische und physikalische Daten und Tabellen, 1906.
2. Bergmann, Über die Grösse des Herzens bei Menschen und Thieren. Inaug. Dissert. München, 1884.
3. Parrot, Über die Grössenverhältnisse des Herzens bei Vögeln. *Zoolog. Jahrb.*, 1894, Systematik, p. 496.
4. Hasenfeld und Romberg, Über die Reservekraft des hypertroph. Herzmuskels, etc., *Arch. f. exp. Path. u. Pharm.*, 1897, xxxix, 333.
5. W. Müller, Die Massenverhältnisse des menschl. Herzens., Hamburg und Leipsig, 1882.
6. Külbs, Exper. über Herzmuskel u. Arbeit. *Arch. f. exper. Path. u. Pharm.*, 1906, lv, 288.
7. Heinz, Handbuch der exper. Path. und Pharm., 1905, i, Part 2, 886.
8. Grober, Untersuchungen zur Arbeitshypertrophie des Herzens. *Deut. Arch. f .klin. Med.*, 1907, xci, 502.

THE PRODUCTION OF AGGLUTININS IN THE ANIMAL BODY BY THE INOCULATION OF SUBSTANCES OTHER THAN PRODUCTS OF BACTERIAL ORIGIN.[1]

By KATHARINE R. COLLINS.

(*From the Research Laboratory of the Health Department of New York City.*)

There is to-day among observers working along the lines of immunity a tendency to no longer consider a given anti-body and the factors producing it as a compact inseparable entity following the laws of protoplasmic continuity, but as a complex body that may be split up into component parts, which may exhibit individuality of structure and independence of action. This view of the subject has been given an impetus by the work of Vaughan and later by Obermeyer and Pick. Vaughan derived split products from bacterial proteids and egg-albumen, representing a poisonous and non-poisonous portion. Obermeyer and Pick found by iodizing protein they so changed it that when the iodized portion was inoculated into animals only non-specific precipitins were formed. This led them to believe that specificity in this case was due to the aromatic radical which was changed by iodization.

The following work was begun in 1905 with hope of determining through the nature of substances having the power to produce agglutinins in the animal a more intimate knowledge of the antibodies and their elaboration in the animal economy. This in a measure was realized. As the work progressed the fact developed that, as far as tested, certain molecules or radicals containing such molecules, are always present in the substances, which induce an increase of agglutinin production; this fact strongly suggests the possibility that these molecules are responsible, for one feature at least, of this phenomenon. The observations arrange themselves under the following heads: (1) Organized ferments, (2) unor-

[1] Read before the American Association of Pathologists and Bacteriologists, April 17, 1908. Received for publication April 30, 1908.

ganized ferments, (3) metabolic products, (4) putrefactive products, (5) inorganic substances.

Organized Ferments.—Ballner and Sagasser in 1904 succeeded in producing, artificially, agglutinins for *Bacillus typhosus* by inoculating animals with red yeast cells (Rosa hefe), but the yeast cells themselves were not agglutinated by the serum. Acting upon this suggestion of Ballner, I inoculated rabbits with living cultures of brewer's yeast. After four or five inoculations the sera of these rabbits were tested with the following organisms:

1. *B. dysenteriæ,* 3 strains (Shiga, Flexner Manila, Park Mt. Desert).
2. *B. typhosus,* 2 strains (Pfeiffer, Mt. Sinai).
3. *B. Coli,* 2 strains (Laboratory, Colon X).
4. *B. paratyphosus.*
5. *B. pyocyaneus.*
6. *B. proteus vulgaris.*
7. *S. choleræ.*
8. *Pneumococcus,* one strain.
9. *Streptococcus,* one strain.
10. *B. mallei.*

The result of inoculations with yeast cell may be shown in the serum of a young goat by the following table.

TABLE I.

	Normal Serum			After 8 Inoculations.					
	10	20	50	10	20	50	100	200	500
Shiga	—	—		—	—				
Flexner Manila	+	+	—	++	++	++	++		
Park Mt. Desert	—	—		—	—	—			
Pfeiffer	+	—		++	++	++	++		
Colon	+	—		—	—				

	After 13 Inoculations.						After 18 Inoculations.						
	10	20	50	100	200	300	10	20	50	100	200	500	1000
Shiga	—						—						
Flexner Manila	++	++	++	++	++	+	++	++	++	++	++	++	++
Park Mt. Desert		—	—	—	—		—	—					
Pfeiffer	+	+					—						
Colon	—						—						

Several rabbits inoculated with yeast cells also gave the same results as the goat. The serum of these rabbits when tested showed a steady increase after inoculation, for the Flexner Manila strain of *B. dysenteriæ,* an increase for *B. typhosus* and sometimes for

Colon X strain of *B. coli* with the subsequent disappearance of the two latter. This disappearance of the agglutinins for *B. typhosus* and *B. coli* would seem to indicate one of two things. First, the normal cells of the rabbit possess a greater potentiality for the manufacture of Flexner Manila agglutinins, on account of the cell being subjected to a stronger and more specific stimulation in this direction and therefore when influenced by non-specific substances the cell forms agglutinins for this organism in preference to others because of this stronger initial stimulation. As the inoculations proceed the increment of production for the Flexner Manila strain becomes more marked and finally the entire energies of the cell seem to be occupied in forming these agglutinins to the exclusion of the others.

On the other hand the possibility suggests itself that the stimulating agent may not be as wholly non-specific as generally considered; this phase would then agree with that seen when definite specific organized agents such as bacteria or their products are used, stimulation of common agglutinins being induced. Common agglutinins may continue throughout to be formed to an equal degree along with the specific, or may disappear almost entirely after long immunization. This disappearance or persistence seems to depend somewhat upon the relation of the heterologous organisms to the homologous. The more nearly the species are related the more persistent the common agglutinins.

I have not been able to identify the Rosa hefe used by Ballner and Sagasser. The red yeast mentioned in most works on fermentation is, strictly speaking, a torula and not a true yeast. Several rabbits were inoculated with the cells of the so-called red yeast obtained from the air. This did not give rise to an increase in the production of agglutinins, though later these same rabbits responded to inoculation of brewer's yeast cells. Several strains of brewer's yeast, differing in their action upon beerwort, were tested without presenting any appreciable variation in results. The brewer's yeasts were obtained in pure cultures from the laboratory of the Brewer's Academy, New York City, and were cultivated on 10 per cent. beerwort agar. Living cells were used. Beerwort alone inoculated into rabbits was without effect.

The serum of the young goat mentioned above was subjected to the exhaustion test with *B. typhosus,* the Flexner Manila organism and living brewer's yeast cells—and cells of the torula. The agglutinins for Flexner Manila were completely exhausted by this organism. *B. typhosus* and the yeast cells absorbed all but five per cent. of the Flexner Manila agglutinins. This amount not absorbed approximately represents the amount of normal agglutinins present in the serum, which on account of its more specific nature would resist the action of purely non-specific agents.

Unorganized Ferments.—The possibility that other enzymes might bring about this increase in the animal of preëxisting agglutinins suggested the use of the unorganized ferments, diastase, pancreatin and invertin. The results obtained by the inoculation of these substances coincide with those obtained by the inoculation of yeast cells.

The agglutinins were in each case increased from 1 : 50 to 1 : 500 after five or six, weekly, inoculations. The enzymes, however, failed to absorb the agglutinins thus raised from the homologous serum.

Products of Metabolism.—Nuclein as a component part of the yeast cell was first used. Vaughan obtained a certain amount of protection against the pneumococcus in guinea pigs by the inoculation of nuclein previous to the pneumococcus infection. The nuclein was used in the form of a nucleoproteid from the pancreas, for which I am indebted to Dr. Levene of the Rockefeller Institute for Medical Research. The other products used of the metabolic group were lecithin and proteoses from egg. The same increase of agglutinins for Flexner Manila type of *B. dysenteriæ* followed the inoculation of these substances as with yeast and enzymes. There was the initial rise for Flexner Manila type, *B. typhosus* and *B. coli,* with the subsequent dropping out of the two latter and a continued rise of the former as the inoculations were continued.

Products of Putrefaction.—The products of putrefaction tested were indol, skatol, ethyl mercaptan and phenol. I am indebted to Dr. C. A. Herter for the indol, skatol and mercaptan, and also for some valuable suggestions as to their use.

Ethyl mercaptan in one per cent. solution produced an increase of agglutinins in two rabbits after the fifth inoculation and followed the same course as in the cases where the enzymes and metabolic products were used. A control rabbit on injection of ethyl alcohol did not show an increased production of its normal agglutinins. Indol and skatol were without effects, as I expected, on account of the readiness with which they combined with the preformed sulphates and were excreted. Phenol, however, did enter into systemic relation with the organism and toxic effects were demonstrated in the animals inoculated, but a rise of agglutinins did not occur after a number of inoculations, the animals finally dying from excessive abscess formation.

At this point two factors present themselves as possible influences in causing an increase in agglutinins. The first is the increased production and destruction of leucocytes and the second is the fact that the substances bringing about this increase of agglutinins with the exception of the enzymes, concerning whose structure little is known, all possess a formula containing phosphorus or sulphur molecules, while those failing to effect an increase do not possess either element.

First the effect of the inoculations of these substances upon the leucocytes may be considered. Dieudonné claims that animals possessing agglutinins for a certain organism may have the amount of agglutinins increased by the inoculation of exciters of leucocytes such as aleuronat and hetol. Aleuronat in our hands brought about an increase of the initial agglutinins similar to that caused by the substances used in the preceding experiments. Hetol was not used.

To test the effect of our inoculations upon leucocytes, blood counts were made upon normal rabbits, rabbits inoculated with substances increasing the agglutinins, and substances which did not affect the agglutinins. The blood counts exhibit the same irregularity in the normal rabbit and in the inoculated rabbits irrespective of the occurrence or non-occurrence of an increase in agglutinins. This irregularity is in accordance with the statement of Brinckerhoff that the number of leucocytes per millimeter in the peripheral blood of the rabbit is constantly changing. It would appear from this irregularity that leucocytes could not account for the increased

production. The action of aleuronat should, therefore, be ascribed to its behavior as a proteid or derivative of a proteid rather than as an excitor of leucocytosis.

Inorganic Salts.—To follow more in detail the suggestion developed by the fact that phosphorus and sulphur were the elements possessed in common by the augmentors of agglutinins, several soluble inorganic salts of sulphur and phosphorus were tested, and their action was controlled as far as practical by salts containing the same base but not the same radical.

Several rabbits were inoculated with sodium phosphate, sodium sulphate, calcium and potassium phosphate. Control rabbits were inoculated with sodium chloride, calcium chloride, and potassium chlorate. A young goat was inoculated with sodium sulphate and a control goat with sodium chloride. The sera of the rabbits and the goat receiving the sulphur and phosphorus compounds showed the characteristic increase of agglutinins for the Flexner Manila strain of *B. dysenteriæ*. The agglutinins for *B. typhosus* and *B. coli* were only slightly stimulated in a few instances. The agglutinins of the control animals inoculated with the salts that did not contain the sulphur or phosphorus molecules were unaffected. The doses varied according to the toxicity of the salts used and the concentrations ranged from one tenth normal to twenty-five per cent. solutions. The concentration of the solutions had no apparent effect upon the results. It is interesting to note that Vaughan found the phosphorus in the non-toxic portion of the split products which is the part that gives immunity.

A question arises as to the character of the action of these substances. Do they merely stimulate to greater activity a specific function of the cell already established or do they possess something in common with the bacteria which admits of their initiating specific action in some degree? An attempt was made to answer this question in the following manner.

Two rabbits each having normal agglutinins for the Flexner Manila type of *B. dysenteriæ* up to 1 : 50, but none that were appreciable for Shiga or Park Mt. Desert types, were inoculated one with the Shiga type and the other with the Park Mt. Desert organism. After three inoculations the sera of these rabbits aggluti-

nated their homologous organisms in dilutions of 1 : 100. The index for the Flexner Manila type remained unchanged. The bacterial inoculations were then stopped and sodium sulphate substituted in one rabbit and diastase in the other. After four or five treatments the sera from both rabbits were tested. The agglutinins for the Flexner Manila organism were increased up to 1 : 500, while those for Shiga and Park Mt. Desert type remained unchanged and later disappeared.

These two experiments are not sufficient, however, to prove or disprove the assumption that the action is one of augmentation and not of initiation. I have mentioned the foregoing question because it suggests several points to be taken into consideration in answering it. First, the influence that has brought about the normal agglutinins in the rabbit for Flexner Manila has been acting practically during the adult life of the animal, while the influence of the Shiga or Park Mt. Desert organisms has only been exerted for a comparatively short period of time; hence the function of the cell to form the Flexner Manila agglutinins would be more permanent than for the other two organisms. Now when the rabbits are inoculated with strong specific substances as Shiga or Park Mt. Desert the cell responds accordingly as long as this stimulus is kept up—but upon removal of this influence and the substitution of a, presumably, non-specific stimulus, then the cell by preference responds in the direction of the more accustomed function of forming agglutinins for Flexner Manila. Another point to be considered is the fact that after withdrawal of the specific influences of Shiga and Park Mt. Desert the cause producing the normal agglutinins for Flexner Manila in the rabbit continues to act, thus adding its influence to the stimulus of the non-specific sodium sulphate inoculations and so determining the direction of the activity of the cells.

At this stage of the present investigation biological rather than physical laws seem to offer the most probable explanation of the production or augmentation of agglutinins which has been described; perhaps stimulation of certain cell activities occurs because some necessary element which enters into the cell or acts as a ferment adjuvant is provided.

The work of several authors on ferments is suggestive by anal-

ogy. Bertrand found that manganese was present in laccase and activity of laccase was proportional to the amount of this salt present.

Calcium salts are found to be essential to enzymes which cause clotting and Magnus has given the name of co-ferment to those substances, but this term has been rejected by Harden and Young. Harden and Young found that boiled yeast which was incapable of initiating fermentation alone when added to unboiled yeast increased its action to a considerable extent. They found this increase due to the presence of arsenates which brought about the same results as the phosphates.

It is too early to draw definite conclusions concerning the effect of various substances upon the production of agglutinins and the suggestions that have been offered are tentative, pending further work which is in progress.

BIBLIOGRAPHY.

Ballner and Sagasser, *Arch. für Hyg.*, 1904, li, 245.
Brinckerhoff, *Jour. of Med. Res.*, 1904, v, 173.
Dieudonné, *Med. Klin.*, 1906, ii, 575.
Harden and Young, *Proc. Roy. Soc., Series B*, 1906, lxxvii, 405.
Obermeyer and Pick, *Wiener klin. Woch.*, 1906, xix, 327.
Vaughan, Cellular Toxins, p. 100; *Jour. of Infectious Diseases*, 1907, iv, 476.

SOME DIFFERENTIAL COUNTS OF THE CELLS IN THE LYMPH OF THE DOG: THEIR BEARING ON PROBLEMS IN HÆMATOLOGY.[1]

By F. PEYTON ROUS, M.D.

Instructor in Pathology, the University of Michigan.
(From the Pathological Laboratory of the University of Michigan.)

Our knowledge of the cell-formula of the lymph is nearly comprised in the statement that small mononuclear elements make it up almost entirely. No systematic differential counts of the lymph-cells in man or in other animals are on record. According to the most recent work, that of Weidenreich (1), whose paper is as yet only reported in brief, one finds in the thoracic duct of the rabbit, the dog, the cat, the guinea-pig and the monkey, non-granular cells in large number, "especially little lymphocytes, but next to these large leucocytes with round nuclei, in which last all stages of mitosis are met. . . . Finely granular leucocytes (neutrophile or amphophile) are few, as are eosinophile leucocytes."

As an aside to another investigation I have had opportunity to observe cells from the lymph of a number of healthy dogs. Differential counts of these have disclosed facts which seem worthy of report.

There exists a good general description of the elements found in the lymph of the dog—that of Biedl and v. Decastello (2). They noted in the fluid all the cells of the animal's blood except the mast-cell. Polymorphonuclear neutrophiles were present only as evidence of blood-contamination. Mitoses in the mononuclear leucocytes were rare. Once they found a large number of eosinophile cells independent of blood-admixture. These authors give no differential counts of the leucocytes. Delamere (3) mentions that out of 133 successive cells he noted 128 lymphocytes, three polymorphonuclear neutrophiles, and one eosinophile.

[1] Received for publication April 22, 1908. Aided by a grant from the Rockefeller Institute for Medical Research.

Lymph for the counts here recorded was obtained by operation on animals anæsthetized with morphine and chloroform. A fine, glass cannula was inserted in the thoracic duct near its mouth, and cover-glass preparations immediately taken, to be colored later with Wright's stain. The counts, which for the most part total five hundred cells—never less than three hundred—were in each case derived from observations on several slips, made from different drops of lymph collected at short intervals. In this way an attempt was made to obtain the average cell-formula, but the individual spreads from the same animal proved to differ little in this regard.

The results are best taken up in the consideration separately of Tallqvist and Willebrand (4), Dawson (5), Busch and v. Bergen (6), render a description of most of these unnecessary. Appended (6) render a description of most of these unnecessary. Appended are complete counts from the lymph of twenty-three dogs.

1. *Polymorphonuclear Neutrophiles.*—These were observed only in the presence of many red corpuscles, and then, with one exception, in such small quantity as the presence of blood in the lymph would quite account for. A little blood in the dog's lymph is practically the rule, as many previous observers have noted. Often its source is discovered in an anastomosis of the thoracic duct with small veins in the superior mediastinum, but occasionally it gains entrance further back in the lymph-system. The white cells introduced into the lymph in this way are few. That the polymorphonuclear neutrophiles in the normal lymph are due wholly to contamination was several times shown with some exactness by quantitative counts of the red and white cells of the lymph and of the blood, conducted with another end in view (Rous (7)). In these cases the ratio between polymorphonuclear neutrophiles and red cells in the lymph coincided with that found in the blood.

In one instance, as noted above (Dog H1), polymorphonuclear neutrophiles were found in an abundance that admixture of blood could not account for—11.5 per cent. of the 4,500 white cells per cubic millimeter of lymph. At autopsy of this animal its organs seemed normal macroscopically. The instance remains unexplained.

2. *Eosinophiles.*—These striking cells were almost always found

in the lymph, and often in considerable numbers,—12 per cent. of the total white cells in one instance. They average 2.6 per cent. Their independence of blood-admixture is shown by their relative abundance when compared with the polymorphonuclear neutrophiles. The finding has a special interest because of the well-known general relationship between the eosinophile and conditions of nutrition, and between the eosinophile and animal parasites. Accordingly steps were taken to find whether an eosinophilia of the lymph depends on these factors.

Heidenhain (8) first pointed out that the intestinal mucosa of the well-fed dog is thronged with eosinophiles. They are most abundant when the animal is on a mixed diet, and become fewer if meat is withdrawn from the food, or if there is overfeeding with it. Starvation causes a profound diminution in the cells. These observations have been confirmed on other animals. Opie (9) has recently shown that when food is withheld from the guinea-pig the number of eosinophiles in its blood, after a transient increase, falls off rapidly.

In the present instance the time that elapsed between the animal's last meal and the operation at which lymph-smears were obtained was purposely varied much. From the diet of half of the

TABLE I.

Series A.—Much Meat in Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Cl	5	Chylous.	11.6	No autopsy.
Hl	27	Thinly chylous.	2.4	One specimen of *Toxacara canis*.
Il	27	Slightly chylous.	7.8	*Dipylidium caninum* 0.5 gram.
Bl	27	Opalescent.	2.3	No autopsy.
Gl	27	Opalescent.	2.5	One specimen of *Toxacara canis*. *Dipylidium caninum*, 0.5 gram.
Ml	27	Opalescent.	6.9	*Tænia pisiformis* 1.7 grams.
Dl	30	Opalescent.	1.0	No autopsy.
Ll	52?[2]	Quite chylous.	2.9	*Dipylidium caninum* 0.5 gram.
Nl	52	Opalescent.	0.4	*Dipylidium caninum* 0.8 gram.
Kl	52	Nearly clear.	1.0	No parasites.
Jl	52	Nearly clear.	1.1	*Toxacara canis* 1.8 grams.

[2] Autopsy shows recent feeding. Stomach and small intestine contain much food.

Series B.—Meat Almost Wholly Excluded from Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Bt	2	Thinly chylous.	0.0	Two specimens of *Toxacara canis*.
C_2l	3	Thinly chylous.	2.8	*Dipylidium caninum* 0.2 gram.
Ft	$3\frac{1}{2}$	Chylous.	2.3	*Tænia pisiformis* 17 grams.
Rl	4	Chylous.	3.3	No parasites.
Ql	20	Clear.	1.3	*Dipylidium caninum* 1.0 gram.
Sl	27	Opalescent.	0.0	*Toxacara canis* 3.0 grams.
Yl	27	Opalescent.	0.0	*Tænia pisiformis* 1.9 grams.
Tl	27	Slightly opalescent.	1.2	*Tænia pisiformis* 16.0 grams.
Zl	27	Clear.	0.6	*Tænia pisiformis* 9.4 grams.
Xl	52	Slightly opalescent.	1.1	*Tænia pisiformis* 11.5 grams.
Pl	52	Clear.	1.6	No parasites.

dogs meat was almost wholly excluded, carbohydrates being substituted. The results are made plain by the following table.

These figures show that a mixed diet rich in meat considerably favors the occurrence of eosinophiles in the lymph, as compared with one of which meat forms a slight part. Furthermore, when all food is withheld the number of eosinophiles in the lymph becomes much fewer. Series A. demonstrates this especially well.

To determine the influence of animal-parasites a record was kept of those that autopsy of the dogs disclosed. Only such as were prominent macroscopically were noted. These were washed, fixed in formalin, and then weighed. The table gives the data resulting. No clear relationship between the presence of parasites and the number of eosinophiles of the lymph is to be seen.

One may well ask, whence come these eosinophiles? Are they the result of a local formation in lymph-glands and intestinal mucosa, or do they represent a migration, more or less indirect, from the blood-stream? Biedl and v. Decastello concluded that the eosinophiles formed in the lymph-glands of the dog must enter the blood directly, since only once did they come upon these cells in the lymph. But the present findings destroy the ground for that conclusion. Simon (10) has noted a few eosinophile myelocytes in the intestinal mucosa of the dog, though not sufficient to account for its content in polymorphonuclear eosinophiles. It is conceded that these, in the main, gather there from the blood. The fluctuation in the lymph's content of eosinophiles agrees remarkably with

that observed by others in the intestinal mucosa, and the fact suggests that this mucosa may be the proximal source of the eosinophiles of the lymph.

3. *Mast-cells.*—These were not seen in the lymph.

4. *Lymphocytes.*—In the study of the dog's blood several classes of these may be separated with reference to staining properties of nucleus and cytoplasm, relative proportion of the two, and size of the cell as a whole (Dawson). But in dealing with the lymph it was soon found a sufficient task to make one group of these cells as distinct from the large mononuclears. Botkin long ago called attention to the effect of pressure as determining the appearance of lymphocytes in spreads, and he held this responsible for much of the individual variation seen. The factor comes into special play in preparations made from the lymph, because this fluid lacks the "body" necessary to the production of uniform films. Frequently cells at the periphery of a lymph spread average twice the size of those at the center.

As a standard to separate large mononuclears from small the size of the polymorphonuclear neutrophile in the same specimen of lymph, or that failing, in the animal's blood, was adopted. A mononuclear element of which the nucleus was smaller than an entire polymorphonuclear neutrophile was classed as a lymphocyte, no matter what its amount of cytoplasm. Such elements constituted from 69.8 to 96.8 per cent. of the total white cells, with an average of 87.6 per cent.

5. *Large Mononuclear Leucocytes.*—Mononuclear elements with a round or oval nucleus larger than that just defined for the lymphocyte were classed as "large mononuclears." Occasional mitoses were observed in them. From 1.8 to 18 per cent. of such cells were found in the lymph, with an average of 5.1 per cent. The relation of these to the like elements in the blood is discussed further on.

6. *Transitional Leucocytes.*—The characteristic transitional cell of the dog's blood is slightly larger than the polymorphonuclear neutrophile. The nucleus is indented, often bilobed or deeply cleft into two or three irregular, blunt branches. With Wright's stain the cytoplasm is a distinct blue, usually of light tint, as compared

with the pink, more or less granular, cytoplasm of the polymorphonuclear neutrophile; and the nucleus is a medium dark blue in contrast with the dense, purplish blue of that of the latter cell. The most complex nucleus of a transitional is thick and clumsy as compared with that of a neutrophile, and the chromatin is much less compact. The cytoplasm occasionally shows a few neutrophile granules. All gradations between this cell and the typical large mononuclear exist, but, as has been brought out, it can always be sharply distinguished from the polymorphonuclear neutrophile.

In my preliminary observations on the lymph transitional leucocytes were but seldom noted, indeed much less frequently than polymorphonuclear neutrophiles. To test this finding, in the later counts all mononuclear elements with indented nucleus (even cells in evident process of division, and those with reniform nucleus) were put under the head of "transitionals." Yet despite this latitude the group remained a small one. In one case (Dog P1) 1.6 per cent. of these cells were present; but altogether, out of 10,511 leucocytes counted, only forty-one were "transitionals." At least half of these forty-one were mononuclear elements in process of division. In the lymph of six of the twenty-three animals "transitionals" were not met with during an average count of five hundred cells.

This is in marked contrast to the frequency of transitional leucocytes in the dog's blood. For the purposes of such contrast special differential counts were made of smears taken at time of operation from the animals yielding the lymph. The counts of the authors already quoted could not be utilized, since in these the transitional and large mononuclear leucocytes are lumped under one head. In the special counts (which are appended in full) cells with a merely reniform nucleus are termed "large mononuclears," and only those with a nucleus more deeply indented are accepted as transitional leucocytes. Thus the class of transitional forms is made radically narrower for the blood than for the lymph. Yet in the blood these cells occur in the proportion of 1 to every 3.1 lymphocytes, whereas in the lymph there is only 1 to every 224 lymphocytes. The table shows this fact.

TABLE II.

Transitional Leucocytes and Lymphocytes.

	Lymph.			Blood.		
Dog.	Transitionals.	Lymphocytes.	Total Count.	Transitionals.	Lymphocytes.	Total Count.
Bl	4	452	526			
Cl	3	477	684	11	78	510
Dl	0	445	502			
Fl	1	469	500	10	47	315
Gl	0	504	550	25	16	311
Hl	0	405	545	21	45	317
Il	0	431	510	15	72	344
Jl	5	332	363	27	51	320
Kl	1	284	303	14	41	306
Ll	1	478	519	21	106	305
Ml	2	457	536	14	69	310
Nl	0	467	492	12	64	400
Pl	5	257	307	9	80	400
Ql	1	261	300			
Rl	1	442	509			
Sl	2	484	500	41	36	500
Tl	1	370	402	19	36	400
Xl	4	358	402	15	60	320
Yl	2	371	400	13	32	306
Zl	3	461	500			
Bt	3	314	335			
C_2l	0	461	526			
Ft	2	226	300			
	41	9,206	10,511	267	833	5,366

Plainly the transitional leucocytes of the blood cannot be furnished to it as such through the thoracic duct. True, it is possible to suppose that the production of transitional forms ceases immediately that the animal is operated upon; hence the scarcity of these elements in the lymph. But the other cells are not so affected, and we have no reason to believe that this occurs. The conclusion seems justified that the transitional leucocytes of the blood do not come to it as such in the lymph. They either develop in the blood from other lymph elements or are derived from another source.

Furthermore, if the cell-character of the lymph represent the general type of contribution made by all lymphoid tissue to the blood, then one is forced to conclude that lymphoid tissue either furnishes transitional leucocytes to the blood under the guise of some other element, or else has no hand in their production.

Are similar conclusions for the large mononuclear leucocyte justified? The counts show the existence of from 1.8 to 18 per

cent. of these cells, with an average of 5.2 per cent. in the lymph of the thoracic duct; and these are, of course, added as such to the blood. Nevertheless, a comparison of the ratios between the large mononuclear leucocytes and lymphocytes in the blood and in the lymph, respectively, gives results similar to, though not so striking as, those for the transitional leucocyte. For this comparison it is necessary to add to the number of "large mononuclears" of the lymph that of the few transitional leucocytes found, because cells with reniform nucleus found in this fluid were classed as "transitionals," whereas in the differential counts of the blood they were called "large mononuclears." Yet, despite this inflation of the number of "large mononuclears" of the lymph through the real transitional leucocytes introduced under this head, the ratio of large mononuclear leucocytes to lymphocytes in the lymph is 1 to 17, as compared with a 1 to 4.4 ratio in the blood.

TABLE III.

Large Mononuclear Leucocytes and Lymphocytes.

	Lymph			Blood.		
Dog.	Large Mononuclear.	Lymphocytes.	Total Count.	Large Mononuclears.	Lymphocytes.	Total Count.
Cl	38	452	526			
Bl	85	477	684	19	78	512
Dl	45	445	502			
El	17	469	500	9	47	315
Gl	20	504	550	3	16	311
Hl	10	505	545	7	45	317
Il	16	431	510	6	72	344
Jl	18	332	363	16	51	320
Kl	6	284	303	15	41	306
Ll	14	478	519	15	106	305
Ml	20	457	536	9	69	310
Nl	19	467	492	20	64	400
Pl	27	257	307	18	80	400
Ql	13	261	300			
Rl	11	442	509			
Sl	14	484	500	13	36	500
Tl	12	370	402	7	36	400
Xl	37	358	402	20	60	300
Yl	19	371	400	13	32	306
Zl	24	461	500			
Bt	8	324	335			
Cyl	17	461	526			
Ft	54	276	330			
	544	9,206	10,511	190	833	5,366

Despite these figures it is not necessary to suppose, as for the transitional leucocytes, that large mononuclear forms develop in the blood from other lymph elements, or else have a source outside the lymph. For, in the first place, the lymph certainly does add to the blood a considerable quantity of large mononuclear cells as such; and, second, a relative accumulation of large mononuclear cells might occur through their persistence in the blood for a longer period than the lymphocytes. Third, in the counts on which this discussion is based, the separation of the large from the small mononuclear elements was accomplished through their difference in size alone—a criterion which permits of large error from the subjective side. The separation of the transitional leucocytes rested on peculiarities of morphology and of tint which could not be overlooked.

7. *Unclassified.*—A considerable number of elements were put under this head. These were for the most part cells distorted

Lymph Counts.

Dog.	Lymphocytes.	Large Mononuclears.	Transitionals.	Polymorphonuclear Neutrophiles.	Eosinophiles.	Unclassified.	Total Counted.	Remarks.
Bl	452	38	4	0	12	20	526	
Cl	477	85	3	4	79	34	684	
Dl	445	45	0	0	5	7	502	
El	469	17	1	1	0	12	500	
Gl	504	20	0	2	14	10	550	
Hl	405	10	0	63(!)	13	54	545	Only slight blood admixture.
Il	431	16	0	7	39	17	510	
Jl	332	18	5	3	4	3	363	
Kl	284	6	1	0	3	9	303	
Ll	478	14	1	1	15	10	519	
Ml	457	20	2	12	37	8	536	Considerable blood admixture.
Nl	467	19	0	4	2	0	492	
Pl	257	27	5	8	5	5	307	
Ql	261	13	1	4	4	17	300	
Rl	442	11	1	6	17	32	509	
Sl	484	14	2	0	0	0	500	
Tl	370	12	1	0	5	14	402	
Xl	358	37	4	0	3	0	402	
Yl	371	19	2	3	0	5	400	
Zl	461	24	3	3	0	9	500	
Bt	314	8	3	1	0	9	335	
C_2l	461	17	0	1	14	33	526	
Ft	226	54	2	3	7	8	300	
	9,206	544	41	126	278	316	10,511	
	87.6%	5.2%	0.39%	1.2%	2.6%	3.0%		

while the smears were being made, owing to the lymph's lack of "body." A few typical "basket cells" were observed.

Résumé.—Eosinophile cells are a frequent constituent of the lymph of the normal dog. Sometimes they are quite abundant: in one case noted they formed 12 per cent. of the total white cells and they averaged 2.6 per cent. When food is withheld from the animal their number becomes much fewer. The kind of food on which the dog has subsisted also exerts an influence, a mixed diet largely of meat favoring eosinophiles, as compared with one mostly of carbohydrates. The finding parallels so nearly that of Heidenhain in his study of the eosinophiles of the intestinal mucosa as to suggest this mucosa as the proximal source of the lymph's content of these cells. A systematic record of the animal parasites in the

BLOOD COUNTS.

Dog.	Polymorphonuclear Neutrophiles.	Eosinophiles.	Lymphocytes.	Large Mononuclears.	Transitionals.	Unclassified.	Total.	Remarks.
C[3]	350	44	78	19	11	10	512	
E[3]	216	22	47	9	10	11	315	Two normoblasts seen during count.
G[3]	248	15	16	3	25	4	311	
H[3]	205	32	45	7	21	7	317	
I[3]	207	39	72	6	15	5	344	Two mast cells and normoblasts seen during count.
J[3]	203	19	51	16	27	4	320	Three normoblasts seen during count.
K[4]	216	16	41	15	14	4	306	
L[4]	141	15	106	15	21	7	305	Two mast cells and nine normoblasts seen during count.
M[4]	195	19	69	9	14	4	310	One normoblast seen during count.
N[4]	271	21	64	20	12	12	400	
P[4]	274	10	80	18	9	9	400	
S[4]	360	38	36	13	41	12	500	
T[4]	289	48	36	7	19	1	400	
X[4]	202	14	60	20	15	9	320	
Y[4]	233	11	32	13	13	4	306	

[3] Smears made just after incision.

[4] Smears made just before operation.

dogs used did not bring out any definite relation between these and the eosinophiles of the lymph.

Mast-cells are not a constituent of the lymph of the normal dog. Polymorphonuclear neutrophiles are only present as a result of blood admixture. Lymphocytes, by which are meant non-granular cells with a round or oval nucleus smaller than an entire polymorphonuclear neutrophile from the same animal, form an average of 87.6 per cent. of the cells.

Typical transitional cells are rare in the lymph. Evidently those of the blood must either develop in it from other lymph-elements or must come from another source than the lymph, and thus from another source than adenoid tissue in general.

Large mononuclear cells—non-granular mononuclear elements larger than the lymphocyte as above defined—average 5.2 per cent. of the lymph's leucocytes.

Owing to the lymph's lack of "body" smears made from it show many distorted cells. Thus the class of "unclassified" elements is rendered comparatively large.

BIBLIOGRAPHY.

1. Weidenreich, *Anat. Anzeiger,* 1907, xxx, 51.
2. Biedl and v. Decastello, *Arch. f. d. gesam. Physiol.,* 1901, lxxxvi, 259.
3. Delamere, "The Lymphatics," by Delamere, Poirier, and Cuneo, trans. by Leaf, Chicago, 1904.
4. Tallqvist and Willebrand, *Skandin. Arch. f. d. Physiol.,* 1899, ix, 37.
5. Dawson, *American Jour. of Physiol.,* 1900–1, iv, 1.
6. Busch and v. Bergen, *Jour. of Med. Research,* 1902, viii, 410.
7. Rous, *Jour. of Exper. Med.,* 1908, x, 238.
8. Heidenhain, *Arch. f. d. gesam. Physiol.,* 1888, xliii, Supplementheft.
9. Opie, *American Jour. of the Med. Sciences,* 1904, cxxvii, 217 and 477.
10. Simon, *Compt. rend. d. l. Soc. de Biol.,* 1905, lix, 648.

OBSTRUCTIVE HYDROCEPHALUS FOLLOWING CEREBROSPINAL MENINGITIS, WITH INTRAVENTRICULAR INJECTION OF ANTIMENINGITIS SERUM (FLEXNER).[1]

By HARVEY CUSHING and FRANK J. SLADEN,
Baltimore.

Plate XXXI.

Despite the ultimate fatality and the failure to secure a post-mortem examination in the case of this unfortunate patient, the interest, from a therapeutic standpoint, of finding, long after the primary infection, the meningococcus still viable in the cerebral ventricles in association with an obstructure hydrocephalus, whereas the spinal fluid retained no trace of organisms, makes it seem desirable to place upon record even this single experience with the intraventricular injection of serum.

History.—A baby six months old was brought by its mother to the children's dispensary of the Johns Hopkins Hospital on the 21st of March, 1908, with the statement that the child had been wasting for four month and its head enlarging.

There was nothing in the parent's story as then related to Dr. J. H. M. Knox, the physician in charge, to indicate that the condition was other than a simple so-called essential hydrocephalus, but owing to the unusual degree of tension of the head and the infant's apparently critical condition Dr. Knox was insistent that some immediate steps be taken to even temporarily relieve the pressure, and the child was brought by him to the surgical operating room where, as will be related, lumbar and ventricular punctures were performed.

On *physical examination* the patient (Fig. 1) proved to be a frail, weak, semi-comatose baby possessing a greatly and somewhat asymmetrically enlarged head with widely separated sutures and fontanelles. The cranial enlargement was the more apparent when contrasted with the emaciated trunk and limbs. The skin over the small body hung in folds; the ribs were prominent; the abdomen was scaphoid. There was a macular rash over the chest and abdomen. Rectal temperature 99.6°; pulse 110; weight 3,900 grms.

There was a convergent strabismus; the pupils were dilated but reacted to light. Conjunctivæ were clear. Ears were negative. No herpes. No erupted teeth; nursing reflex active.

[1] Received for publication May 15, 1908.

The head was large, measuring 47 cms. in its greatest circumference. The fontanelles were widely opened and tense; in the center of the anterior fontanelle lay an island of bone measuring about 5 cms. in diameter (Fig. 2). All of the palpable sutures were separated. The tense scalp was covered with greatly dilated and tortuous vessels. There was a network of dilated venules in the eyelids. The general appearance of the head was that of infantile idiopathic hydrocephalus.

The head was markedly retracted; neck very stiff and resistent to passive movement; no arching of spine. There was a coarse tremor and marked spasticity of all four extremities. The arms were held flexed at elbows in "driving position"; the legs were occasionally (more usually the left) held flexed at the knee and ankle, but a slight stimulus would cause them to straighten out with accompanying dorsal flexion of the great toes.

The deep reflexes were all exaggerated; Babinsky's toe phenomenon was bilaterally positive. Kernig's sign was not definite; the limbs could be easily straightened by using a little force.

Thoracic and abdominal viscera were practically negative.

Lumbar and Ventricular Puncture No. 1.—Only a small amount of clear fluid was obtained from the lumbar meninges—not enough to diminish appreciably the intracranial tension; consequently a left ventricular puncture was performed at the outer angle of the open fontanelle, 120 c.c. of clear fluid being removed. Cultures were taken from each specimen, and smears were immediately examined, the lumbar fluid showing no organisms, whereas the ventricular fluid contained an abundance of Gram-negative, intracellular diplococci. The cultures from the lumbar fluid remained sterile, whereas those from the ventricular fluid gave a diffuse heavy growth, which proved to be a pure culture of the *Diplococcus intracellularis meningitidis* (Weichselbaum).

Not until the smears were examined did we for a moment suspect that there had been a previous meningeal infection—far from anticipating the actual presence of living organisms. In consequence of this disclosure the child was admitted to Prof. Barker's service, where, in the light of our acquired information, the following somewhat more detailed history (indicative of an acute illness, probably meningitis) was extracted from the parents.

Family History.—Unimportant.

Past History.—This, the fourth child, was born at full time after an easy labor. The head was not large at birth. For the first three months the child was breast-fed; since then, under a physician's direction, it had been put on a variety of changeable diets.

Present Illness.—Four months ago the child, then a seemingly normal baby of only eight weeks, awoke early one morning evidently suffering from some derangement of acute onset. This was followed an hour later by a severe convulsion which produced unconsciousness. On the following day there occurred four or five other convulsions, and again three weeks later still another of the same character, though there had been none in the interval. For several days and nights after the onset the child was feverish and screamed constantly. The eyes are said to have become crossed, the neck stiff and the head drawn back. There was no continuous eruption; no herpes.

The child had been treated during these four months by various physicians for pneumonia, muscular rheumatism, indigestion, intestinal rickets, etc.

In addition to the notes on the child's physical condition given above, a blood examination was made after entrance showing white blood corpuscles 17,000, polymorphonuclears predominating; red blood corpuscles of variable size; none nucleated.

March 22.—Lumbar puncture No. 2 was performed, only 2 c.c. of fluid being obtained: no evident increase of tension. Fifteen c.c. of antimeningitis serum (Flexner) were injected, causing evident discomfort. Microscopically the sediment of the centrifugalized fluid showed no organisms and hardly any cellular elements—only a few leucocytes were found. The cultures remained sterile, confirming the observation made on the fluid removed the day before at *Lumbar Puncture No. 1.*

March 24.—Child seems somewhat better, taking nourishment well. Fontanelles remain less tense than when first seen, that is, before first ventricular puncture. Child lies quietly on its side with head retracted and eyes widely open. Does not appear to see, though pupils react to light.

March 28.—The tension has gradually increased until the fontanelles have become fully as tight as on admission; circumference of head 46.5 cm. *Ventricular Puncture No. 2.* No anæsthesia; needle introduced through outer angle of fontanelle into left ventricle, presumably through second frontal convolution. Clear fluid, unstained with blood, rose in capillary tube to height of 95 cm., though child was quiet without crying or straining. Tube was lowered and 150 c.c. of clear, limpid fluid containing a few flucculi were slowly withdrawn; 15 c.c. of antimeningitis serum were then injected. Child's condition was unaffected one way or another; no vomiting followed the puncture as on the first occasion.

The fluid contained intra- and extracellular diplococci in considerable numbers. There was a diffuse growth on glycerine agar from two loops of the centrifugalized sediment.

The rectal temperature rose, after the puncture, to 101.2°, but the general condition on the following day seemed somewhat inproved.

March 30.—After atropine instillation a satisfactory view of the eye-grounds was obtained for the first time on this day, Dr. Bordley finding a choked disc of at least 3 D. on the right and 2 D. on the left, with extremely large and tortuous veins near the discs.

March 31.—Ventricular Puncture No. 3. Though the fontanelles are somewhat less tense than before and the child evidently gaining, the puncture was repeated; ventricular tension was not measured; 100 c.c. of fluid were withdrawn from the right ventricle and 15 c.c. of antimeningitis serum introduced.

The head measured 46 cm. before tapping, and 45.5 cm. afterwards, showing its ready collapsibility.

The fluid obtained was clear, though yellowish in tint, evidently stained by serum. A centrifugalized specimen showed diplococci in undiminished numbers, though possibly there were more intracellular and fewer extracellular organisms than before. Smears from two loops of the sediment again gave a diffuse growth on glycerine agar.

For two hours after the tapping clear fluid leaked from the puncture wound; the bandage and pillow were stained yellow and it was thought that much of the serum might have been lost. The head became so greatly collapsed that the thin shells of bone overlapped at the sutures.

April 1.—The general condition seems much improved; cheeks and body in general seem to be filling out. Actual gain in weight, however, is not great, possibly owing to loss of fluid. Head gradually refilling.

April 3.—Child irritable; crying much; head again tense, measuring 46.5 cm. *Ventricular Puncture No. 4.* Fluid rose in capillary tube only 50 cm.; 60 c.c. removed and 15 c.c. of antimeningitis serum introduced through needle.

The fluid was clear with no trace of color to suggest serum, much of which may have leaked out after the injection of March 31st. Smears showed diplococci both in and out of cells though greatly diminished in number. On culture two loops of the centrifugalized sediment gave isolated colonies instead of a diffuse growth as before.

April 5.—The rectal temperature again showed a transient rise to 101.8° after the puncture of two days ago. General condition, however, seems to be much improved. Weight 3,970 grm.

April 6.—Weight 4,150 grm. Child doing splendidly; sleeps well, takes nourishment greedily. Head less tense than on any previous occasion 48 hours after puncture. Condition thought to be so favorable that the contemplated retapping of the ventricle was postponed.

April 8.—During the early morning hours, from some unaccountable cause, the patient became cyanotic and developed Cheyne-Stokes respiration. When seen at 7:30 A. M. the baby was in a state of collapse; in a cold sweat; eyes sunken; pulse weak and hardly perceptible; respiration gasping.

The fontanelles had become tense during the night, and as a last resort a *ventricular puncture* (No. 5) was made, in the hope of relieving the pressure symptoms; 140 c.c. of clear, slightly yellowish fluid were removed. The child's condition was not appreciably changed by the procedure, and in spite of stimulation the pulse and respiration gradually grew weaker and the child died six hours later. The rectal temperature did not rise above 99°.

Cultures taken in the routine fashion (two loops of centrifugalized sediment on glycerine agar) from the fluid at this last tapping gave only eight to ten colonies. The smears showed a great diminution in the number of organisms. Some polymorphonuclear leucocytes contained indistinctly staining Gram-negative diplococci; there were very few extracellular organisms to be seen.

Comment.—It has been our impression that the fatalities in many of the cases of cerebrospinal meningitis that we have seen during the past few years have occurred through the medium of cerebral pressure rather than as the result of an overwhelming intoxication or terminal infection. Although not fully dwelt upon in the autopsy notes of each case, in most instances nevertheless the existence of a ventricular dilatation has been recorded. Unfortunately it has not been an invariable custom to harden the brains in situ, and consequently the full extent of the pyo-hydrops ventriculorum may in some cases have escaped notice.

As early as 1898 attempts were made in the Johns Hopkins Hos-

pital to treat some of the more desperate cases of cerebrospinal meningitis by surgical measures. At that time a few patients[2] were subjected to a lumbar laminectomy and a permanent drain was established after washing out the subarachnoid space as thoroughly as possible by retrograde irrigation. One of these patients recovered from the meningeal infection to die of pyo-nephrosis some two months later; another after a period of apparent improvement succumbed to the infection, and the meningeal spaces and basal cisternæ were found post-mortem so occluded with a fibrinoplastic deposit leading to an internal hydrocephalus that it was impossible to conceive of a lumbar drain helping the condition in any way.

In view of these and similar experiences a median suboccipital drainage with opening of the exposed posterior cistern has more recently been adopted and has seemed to promise more than did these lumbar operations; nevertheless, even in these cases, an obstructive hydrocephalus may develop. For this reason during the past two years a number of patients with evident ventricular obstruction have been subjected from time to time to ventricular aspiration. Some few of them have for the time being been markedly improved, although without exception they were all practically in extremis at the time of the operation. The brief history of one of these cases may be cited in illustration.

The patient, a sailor, was brought to the Johns Hopkins Hospital from a North German Lloyd steamer on January 19, 1907. During the voyage another sailor had died from meningitis. The patient was exceedingly ill with the characteristic symptoms of cerebrospinal fever. From the time of his admission to February 7 repeated lumbar punctures—twelve in all—were performed. After the earlier punctures there was considerable improvement; after the later ones none, and finally no further fluid could be obtained. A double choked disc appeared, complete nerve deafness, strabismus, delirium, etc. A cranial operation with puncture of the lateral ventricle at Keen's point of election was performed by Dr. Sowers. There was an almost immediate and a very marked improvement in the patient's condition; he regained consciousness, the choked disc and headache subsided, the temperature became normal, and there were no further untoward symptoms until February 15, when there was a sudden and unexpected exodus.

At the autopsy it was evident that the infection had largely subsided, leaving,

[2] Some of these cases were recorded by Dr. Osler in his Cavendish Lecture. *West London Medical Journal*, 1899, iv, 145.

however, a thick membrane which effectually closed the outlets for ventricular fluid in the neighborhood of the fourth ventricle. The lateral ventricles which were considerably dilated (Fig. 3) contained sero-purulent fluid. The lining surface, particularly of the left ventricle, showed the characteristic granular ependymal inflammation.

Before the days of serum treatment the experiences with lumbar puncture, as in this instance, usually proved symptomatically beneficial up to a certain period of the illness, at which time the punctures would often fail even temporarily to give relief, supposedly owing to the small amount of fluid, if any, which could be obtained. Since the serum injections following lumbar puncture have become a routine with us in the treatment of this disease, its course has almost always been less severe and it seems unquestionable that there has been less of a fibrino-plastic exudate than in the cases which were treated in earlier days by a lumbar puncture alone. This would make it seem probable that under the serum treatment there is a lessened likelihood of the particular complication we are considering.

However, even among our later cases there have been a certain number of fatalities, evidently due to a ventricular block, and in all instances in which an autopsy has been permitted internal hydrocephalus has invariably been found. Such a stasis of ventricular fluid in the closed skull of an adult leads naturally to critical pressure symptoms, and in the reduced physical condition of these patients the complication is often fatal. It is notable too that these signs of cerebral compression—the stupor, increased headache, the choked disc and so on—may supervene with considerable abruptness in the course of an infection which seemed to be progressing favorably, and not uncommonly late in the disease. In an infant, on the other hand, as in this patient the history of whose illness has been detailed, the pressure effects may be warded off through the possibility of cranial enlargement owing to separation of the sutures.

In case, therefore, the acute pressure symptoms can be alleviated, either by operative measures or, in an infant, by the method of relief through cranial distension, it is quite conceivable that the dilated ventricles may continue to hold organisms which remain a menace for a considerable period of time. For this to occur there

presumably must be a discontinuity between the ventricular cavities and the subarachnoid spaces of the brain and spinal cord. So far as clinical evidence can be relied upon, it certainly appears to be conclusively shown in this patient that such a discontinuity existed and that the organisms had completely died out of the spinal meninges and had retained their viability within the ventricles alone.

Late complications, associated oftentimes with a low grade of hydrocephalus and accompanied by irritative symptoms due to cortical changes and to cortico-meningeal adhesions, have in the past not uncommonly been seen after recovery from meningitis—complications which it is to be hoped will be greatly lessened under a more widespread use of serum treatment. It is but natural that they should be seen more frequently after recovery from the disease in childhood than in adult life, and it is well known that a low grade of persistent ventricular distension is not incompatable with subsequent and even unusual mental vigor, provided there has occurred a reopening of the outlets for the escape of fluid.

We have seen a number of cases in which, for some months after the primary infection, symptoms of ventricular distension recurred with a more or less definite periodicity, the pressure symptoms being absent in the interval. In some patients indeed, even after an interval of freedom, they have recurred and persisted. Some of these cases have been subjected to operation and one of them in particular we wish to mention:

The child had been desperately ill for six months during the epidemic of three years ago in New York, and his recovery was long despaired of. Blind and deaf, totally paralyzed for many weeks, there was a gradual slow restoration to fairly normal health. The child, however, began to have frequent convulsive seizures, many of them Jacksonian in character, and also began to fail mentally. An ophthalmoscopic examination showed a low grade of choked disc. A bilateral exposure of the hemispheres disclosed the presence of innumerable fine adhesions between the pia-arachnoid and overlying dura. These adhesions were separated over both hemispheres, the ventricle was aspirated, and at the same moment a lumbar puncture was performed. On lowering the capillary tube used to measure the tension of the fluid in the lumbar region, fluid was withdrawn in large amounts, evidently escaping from the ventricles by this lumbar route, for the somewhat protruding brain subsided markedly. It was the operator's impression that with an open skull the atmospheric pressure against the hemisphere was sufficient to allow a rechanneling of the membranous thickenings over the fourth ventricle, which enabled the pent up fluid to again find

its way from the ventricles into the subarachnoid spaces and thus to escape by its normal channels. The child completely regained its normal health.

This history is cited merely to emphasize the importance of these intracranial complications even in cases of recovery from cerebrospinal meningitis and the possibility of their at least occasional betterment through surgical measures.

Noteworthy in the case the subject of this report are the following facts—namely, the obstructive hydrocephalus; the long-standing ventricular infection with the meningococcus in the presence of sterile fluid in the spinal meninges; and finally the marked diminution in number of the organisms in the ventricles as the result of serum injection even in a case of such long standing.

The case suggests that it may be advisable in other instances of ventricular obstruction in the acute stages of the disease to perform, even in the adult, ventricular punctures and, if organisms are present, to administer serum, with proper precautions, directly into the ventricle.[3] It is possible that in this way the mechanical factors at work in producing the compression may be relieved for a time sufficiently long to tide over the period of plastic inflammation about the brain stem and thus to enable the channels of exit for the fluid from the ventricles again to become reopened.

[3] Unusual care, of course, particularly in the closed skull of the adult, must be observed not only in the ventricular aspiration but more particularly in the reintroduction of serum; for it would be even more hazardous in the case of the ventricle than in the lumbar subarachnoid space to introduce more fluid than has been removed. Flexner has wisely cautioned against this.

In the infant the ventricle may be safely entered through the outer angle of the open fontanelle at a point about 2.5 cm. from the median line. In the adult, on the other hand, cranial penetration is necessary, and in all of our cases, except in the instance cited in this report, Kocher's point of election has been chosen (Fig. 3). There is no especial difficulty in the procedure. A small patch of scalp is shaved and a short one-inch linear incision made about 3.5 cm. from the mid-longitudinal line and about 5 cm. anterior to the Sulcus centralis; the bone is exposed and penetrated by a Doyen perforator followed by a burr, which leaves a cup-shaped fossa and gives sufficient exposure of the dura to assure the operator that there is no large underlying cortical vessel. The hollow exploratory needle, which should have a blunted point with openings upon the side, is then gently inserted into the second frontal convolution perpendicular to its surface, and at a depth of from 4 to 5 cm. readily finds the ventricle, particularly if it is distended. (Cf. chapter in Keen's "System of Surgery," 1908, vol. iii, p. 117.)

EXPLANATION OF PLATE XXXI.

FIG. 1. Photograph showing general appearance of the child. Note enlargement of head and dilatation of superficial vessels, the retraction of the neck with no arching of the spine, the hypertonicity of the muscles and tremor of the extremities, the dorsal flexion of the great toes.

FIG. 2. Photograph of top of head showing separation of sutures and widely opened anterior fontanelle containing isolated island of bone. Note dilatations of vessels.

FIG. 3. Showing degree of ventricular dilatation in fatal adult case of cerebrospinal meningitis. Section of brain after hardening in situ by carotid injection of formalin. Arrow shows direction of ventricular aspiration at point of election.

FIG. 1.

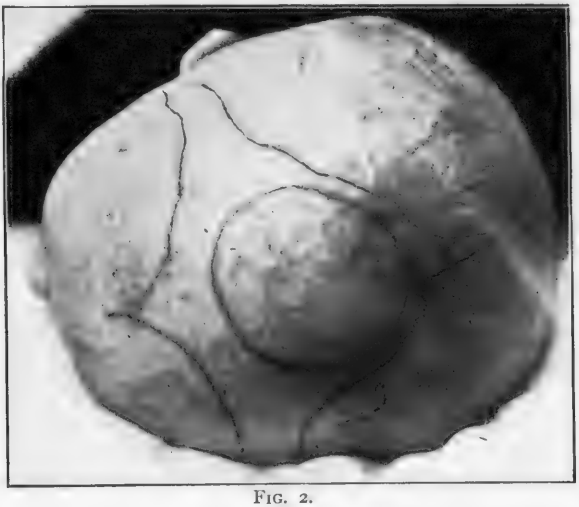


FIG. 2.

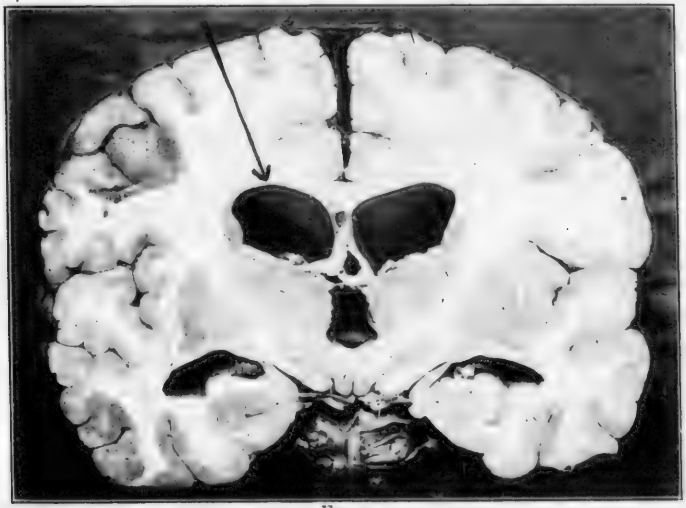


FIG. 3.

ON GLYCOTHIONIC ACID.

By P. A. LEVENE and W. A. JACOBS.

(*From the Rockefeller Institute for Medical Research, New York.*)

Of all the substances belonging to the class of glycothionic acids only one has been the subject of detailed chemical analysis—namely the one discovered by Mörner,[1] first carefully studied by Schmiedeberg and later by Neuberg and Orgler[2] and by S. Fraenkel.[3] As yet it is uncertain whether or not all the substances of this group are identical or different in their chemical structure.

In the present communication a brief report of the analysis of the acid obtained from tendo-mucin and of that obtained from the spleen is given.

It was found possible to obtain a barium salt of the acid possessing a constant composition, which could be best expressed by the formula $C_{14}H_{19}NO_{14}SBa + H_2O$.

	Calculated for this Formula.	Found.
C	27.30	27.29
H	3.43	3.64
N	2.29	2.58
S	5.05	4.85
Ba	22.34	21.90

The analysis of this barium salt has made it probable that its molecule is composed of sulphuric acid, acetic acid, glycuronic acid and aminoglycuronic acid. This conclusion was arrived at on the following grounds.

On hydrolysis with hydrobromic acid with simultaneous oxidation with bromine there was obtained a silver salt with the properties of saccharic acid. The silver calculated for the formula $(HCOH)_4(COOAg)_2$ and the amount found were:

	Calculated.	Found.
Ag	50.94	50.44

[1] *Skand. Arch.*, 1889, i, 210.
[2] *Zeitsch. f. physiol. Chem.*, 1903, xxxvii, 407.
[3] *Liebig's Ann.*, 1907, cccli, 344.

This can be regarded as an oxidation product of glycuronic acid.

Besides, there was obtained a barium salt which had the composition of a mixture of equal parts of aminoglycuronic acid and of glycuronic acid.

	This mixture Requires	Found.
C	27.58	27.27
H	3.64	4.50
N	2.68	2.47
Ba	26.29	25.77

On hydrolysis of the substance in autoclave at 175° C., a silver salt of a substance was obtained which possessed the composition of that of the monoacetylglycuronic acid.

	This body Requires	Found.
C	21.43	21.52
H	2.45	2.40

On hydrolyis with 25 per cent. sulphuric acid in autoclave a silver salt of acetic acid was obtained. That the substance contains no hexose one is justified to conclude from the fact that no levulinic acid was obtained on heating the substance with 25 per cent. sulphuric acid and on the ground that a phenylosazon is obtained from the products of hydrolysis of the substance with great difficulty. This osazon had a melting point = 155° C.

There is also no ground to believe that the substance contains in its molecule a pentose, since on distillation with hydrochloric acid a quantity of furfurol is obtained which does not correspond to that required by the presence of a pentose.

The substance is levorotatory. The barium salt of the substance obtained from the spleen differed in its composition from that of the tendo-mucin.

	Tendo-mucin.	Spleen.
C	27.29	29.74
H	3.64	3.82
N	2.58	3.64
S	4.85	....
Ba	21.90	13.89

THE HUMAN SPLEEN AS AN HÆMATOPLASTIC ORGAN, AS EXEMPLIFIED IN A CASE OF SPLENOMEGALY WITH SCLEROSIS OF THE BONE-MARROW.[1]

By J. L. DONHAUSER, M.D.

Resident Pathologist, Pennsylvania Hospital, Philadelphia.
(From the Ayer Clinical Laboratory, Pennsylvania Hospital.)

PLATES XXXII AND XXXIII.

This paper has been written for the following reasons:

1. To present a case of splenomegaly with very unusual pathological findings.

2. To show the similarity which may exist between bone-marrow changes and splenic transformation, and the relation of the one to the other.

3. To consider certain types of splenomegaly as being compensatory processes dependent upon and due to bone-marrow disease.

History of the Case.—The patient, Samuel K., an oiler, aged 58 years, was brought into the Pennsylvania Hospital, August 5, 1907, in the ambulance during the service of Dr. J. C. Wilson, whom I wish to thank for the following notes.

He complained of malaise, weakness, dyspnœa, shortness of breath, insomnia, loss of appetite, nausea, constipation and œdema of lower extremities. His father died of "debility"; his mother, as result of an accident. Three brothers and two sisters are dead, the causes unknown. Three brothers and three sisters are living and well. There has been no chronic illness in family. The patient was born in County Dongon, Ireland. He lived there until he was thirteen years old; he came to the United States when fourteen, worked on a farm for three years and then was employed by Traction Company as driver and conductor for twenty-three years. He was employed as "workman on road" up to 1902. Since then he has done odd jobs. During the last two years he has been employed as an oiler in an engine room. He has always been a healthy individual. He had measles, mumps and whooping cough when a child. Patient states that as far as he knows he has never been sick before the present illness (with the exception of diseases of childhood). There is no history of malaria, syphilis or gonorrhœa. He drinks a fair amount of tea and coffee. He occasionally drinks

[1] Read before the Pathological Society of Philadelphia, March 12, 1908. Received for publication April 3, 1908.

whiskey and gin as well as considerable beer—15 to 20 glasses a day. He has taken no liquor for past three years.

Patient thinks that his illness began about the first of this June, 1907, with a swelling of the lower extremities, dyspnœa and shortness of breath which gradually increased; he was unable to sleep or lie down with any degree of comfort. Appetite was poor; he was nauseated and vomited once or twice; bowels were regular; there was no cough nor pains in chest. There was occasionally slight vertigo. There was no abdominal pain, distension or tenderness. Patient worked until the middle of June when he was compelled to stop as his condition was becoming worse. He went home and remained there but did not go to bed. Symptoms gradually became more marked; his feet and legs became more œdematous, dyspnœa and shortness of breath increasing with slight precordial pain; appetite became poorer. He began to notice that the abdomen and scrotum were becoming swollen. He was unable of late to lie down on account of dyspnœa. Sleep was very poor. On admission to the hospital temperature was 99° F.; pulse, 108; and respiration, 36.

Physical examination shows a fairly well developed though emaciated man, past middle age. He sits up in bed and is unable to lie down. He breathes with difficulty and is swollen and anemic looking. Abdomen and extremities are moderately swollen and œdematous and pit on pressure. Pupils are equal and react to light and accommodation. Conjunctiva is clear though anæmic looking. The tongue is moderately coated, moist and not tremulous; the breath is offensive. There are some pulsations of vessels of neck. There are no enlarged glands; no rigidity. Brownish pigmentation is present at the root of the neck. Chest is fairly well developed and symmetrical; expansion is poor. At the base of both lungs there are some crepitant and mucous rales, with slight impairment to percussion; breath sounds are somewhat suppressed and distant, but otherwise the lungs are clear. There is normal vesicular breathing with no impairment of resonance; vocal fremitus is fair.

The area of cardiac dullness is slightly enlarged, extending to the upper border of the third rib and 3 cm. to the right of the mid line; the heart beat is circumscribed and moderately forcible. There is also seen and felt just at the edge of costal margin 1.5 cm. to left of the mid line, an impulse. Heart sounds are heard fairly well at all the cardiac areas; they are somewhat forcible but not clear and distinct, and seem to lack muscular tone. At apex there is heard, at times, a soft systoic murmur transmitted to the base. The pulse is moderately rapid, fairly soft and compressible and at times slightly irregular. The arteries are slightly sclerotic. The abdomen is quite distended and tense and pits on pressure in the lower quadrants. On percussion there is dullness in the flanks, and the lower quadrants are slightly tympanitic below costal angle; there are definite succussion splash and movable dullness. There is a large mass in left upper and lower quadrants, apparently projecting from below the costal margin. It is quite hard, apparently solid and smooth, and tender to palpation. The mass extends from the costal margin into the pelvis, approximately measuring 23 cm. and from the median line to left flank, 23 cm. It does not move with respiration and moves only slightly on change of position. Coils of intestine can be felt floating over the mass. Liver reaches from the fourth rib in mid-clavicular line to the costal margin and is tender on palpation. There is no pulsation. The

mass in the left quadrant is apparently the spleen or else is fused with it. Pelvis and scrotum are somewhat swollen and œdematous. There is moderate œdema of the lower extremities.

On admission the hæmoglobin was 85 per cent. (Dare). The blood count was as follows:

Leucocytes, 11,550 per cu. mm.

Polymorphonuclear leucocytes	71.6 per cent.
Large mononuclear leucocytes	13.2 per cent.
Small mononuclear leucocytes	8.4 per cent.
Transitional leucocytes	6.0 per cent.
Eosinophile leucocytes	0.4 per cent.
Basophile leucocytes	0.4 per cent.

Urine is reddish yellow, acid, sp. gr. 1023; there is a large amount of albumin; no sugar; hyaline and granular casts, a few leucocytes, epithelial cells, and mucous cell detritus are found.

Patient improved for a time but gradually became worse and died of asthenia September 10, 1907. After the first day the temperature never advanced above normal point.

The blood was examined from time to time while the patient was in the hospital but apart from the fact that there were evidences of a gradual decrease in the hæmoglobin, a slight decrease in the number of red blood corpuscles and a moderate increase in the large mononuclears, the blood picture showed nothing unusual. The lowest percentage of hæmoglobin was 50 per cent.; the minimum number of red blood cells 3,220,000; of white blood cells 8,950. The differential counts were suggestive of nothing. At no time in the course of the disease were nucleated red blood cells found.

An autopsy was performed seven and one half hours after death. The report is as follows:

A. 1013 D. The body is that of a well built, rather poorly nourished adult male, measuring 155 cm. in length. Rigor mortis is present to a slight degree. Bluish red discoloration of dependent parts. Pupils are equal, clear and widely dilated. No lymph glands palpable. External genitalia apparently normal. The abdomen is protuberant.

Abdominal cavity contains approximately 500 c.c. of a clear straw-colored fluid. The bowels are in a collapsed condition and the omental fat is scanty. The parietal and visceral peritoneal surfaces are smooth and glistening with well-injected vessels. The mesenteric glands are enlarged. The appendix is directed downward, inward and upward in the right iliac fossa: it measures 5 cm. in length, is patent throughout and apparently normal. The spleen is seen to extend 5 cm. below the costal margin.

Each pleural cavity contains approximately 1,000 c.c. of a yellow-brown, clear fluid. There are a few adhesions over the right pleura; the left lung is bound to the chest wall by numerous adhesions. The pericardial cavity contains about 50 c.c. of a clear straw-colored fluid. The visceral pericardium is covered, in numerous areas, by large white plaques which are firmly adherent to the underlying tissue.

Heart.—Weight, 450 grm. The right ventricular wall measures 8 mm. in

thickness, the left ventricular wall 3.25 cm. in thickness. The heart's flesh is of a pale red color, very firm in consistency and there is evidence of an increase in the interstitial connective tissue. The coronary arteries are patent throughout and free from sclerosis. Tricuspid, aortic and pulmonic valves are negative. The anterior leaflet of the mitral valve is somewhat hard in consistency and apparently slightly thickened. Five millimeters above one of the aortic cusps is a calcareous area measuring 1 cm. in diameter.

Lungs.—The right lung is voluminous, crepitant in some portions and boggy in other areas. It is of a purple color. No nodules are felt. On section a grey fluid exudes from a cut surface which is of a gray-black color dotted with small areas of a dark red. A few white fibrous plaques are found in the right pulmonary artery. The left lung is somewhat smaller than the right one and is tougher to the feel. The external surface is purplish grey in color. On section the upper lobe resembles the right lung. The lower lobe is, however, entirely collapsed and is of a dark red-brown color; on pressure it exudes a slight amount of frothy red fluid. There are a few sclerotic patches in the left pulmonary artery.

Mesenteric glands are greatly enlarged throughout and on section present areas of caseation and calcification.

Spleen.—22 x 15 x 8.5 cm.; weight, 1,470 grm. Its external surface is covered with small and large fibrous tags. The organ is very firm and regular to the feel. Small whitish plaques, varying in size from that of a millet seed to a split pea, are noted over its entire surface. On cut section the capsule is seen to be somewhat thickened and the organ, as a whole, of a dark red color. The trabeculæ are very distinct. The Malpighian bodies are barely visible. Scattered throughout and indiscriminately arranged are small, rather soft areas of a light red color, spherical in form, varying in size from a pin point to that of a large bean. These soft nodules stand out prominently as light red areas in contradistinction to the surrounding tissue which is of a much darker red. The above described portions do not appear to be encapsulated, though they are definitely spherical.

Kidneys.—The right kidney measures 8 x 6.5 x 4 cm. The capsule strips quite readily though it is slightly adherent in areas. A small retention cyst is noted. On section the cut surface is gray-red in color. The cortex measures 8.5 mm. in thickness. The glomeruli are rather prominent; the pyramids and striæ very distinct. The pelvic fat is normal in amount.

The left kidney measures 10 x 4.5 x 3 cm. It is somewhat lobulated and rather soft in consistency. The capsule strips with difficulty. On section the cut surface is gray-red in color. There is an evidence of confluency of cortex with the pyramids, no distinct line of demarcation being seen. The glomeruli are quite distinct, as are also the striæ and pyramids. There is a small cortical retention cyst.

Liver.—23 x 12 x 9 cm.; weight, 2,130 gms. It is very hard to the feel. The external surface is free from adhesions and is of a pink-gray color. The edges are slightly rounded. On section the cut surface is reddish yellow in color, studded with small patches of yellow-red, especially noted toward the center of the liver lobules. There is a marked engorgment of the capillaries. The portal connective tissue is apparently slightly increased in amount. The bile ducts are patent; no stones are found. Vessels are apparently normal.

Gall bladder contains approximately 50 c.c. of a viscid brown fluid.

Adrenals are apparently normal.

Urinary bladder is apparently normal.

Testicles are somewhat œdematous but there are no signs of orchitis. There is a small cystic tumor of the right epididymis.

Prostate and seminal vesicles are apparently normal.

Aorta is apparently normal.

Gastric mucosa shows congestion and is covered with thick mucus; otherwise it appears normal.

Pancreas is apparently normal.

The intestinal mucosa especially in the neighborhood of the big gut shows a marked hyperplasia and is covered by thick mucus such as is seen in the stomach. In the neighborhood of the jejunum there is marked vascular injection.

Bone marrow removed from the middle portion of the right femur shows a very firm dark brown-red tissue.

Anatomical Diagnosis.—Acute œdema of lungs. Dilatation and hypertrophy of right and left ventricles. Chronic myocarditis. Chronic splenic tumor. Atelectasis of lower lobe of right lung. Chronic parenchymatous nephritis. Cloudy swelling of liver and kidneys. Tuberculosis of mesenteric lymph glands. Sclerosis of bone marrow. Fibrinous pleurisy.

MICROSCOPICAL EXAMINATION.

Heart.—Muscle fibers are of normal size but are quite granular and contain a fair number of vacuoles. There is some increase in the interfibrillar connective tissue.

Lung.—Pleura is somewhat thickened. Many of alveoli are filled with a serous-like material. Capillaries are engorged; no large giant cells are seen.

Liver.—Capsule shows moderate thickening. The liver cells are swollen and granular and show a well advanced grade of vacuolization. Some areas show so high a degree of granulation that the liver cords have completely lost their contour and appear piled upon one another. Extending from the center of many of the lobules and in some portions almost to the periphery, are areas of necrosis with complete disappearance of the liver parenchyma; they have been filled with red blood corpuscles, vast numbers of polymorphonuclear leucocytes and an occasional round cell. The connective tissue around the bile ducts shows slight thickening with round cell infiltration. The periportal connective tissue is somewhat increased. The inter- and intralobular connective tissue does not appear to be increased in amount. The blood vessels and capillaries are filled with red blood cells. Their walls are normal. No multinucleated or large vesicular cells are found in any sections.

Kidneys.—Attached to the cortex are bands of connective tissue which show a moderate degree of round cell infiltration. At the juncture of the cortex with these fibrous bands, are large clusters of round cells. Many of the glomeruli appear normal in all respects, others are atrophied and still others have undergone a complete hyaline change. The epithelium of the tubules is swollen and granular and many of the lumina are almost occluded by the swollen and desquamated cells. There is a slight increase in the intertubular connective tissue. The blood-vessel walls are slightly thickened. The capillaries and larger vessels are filled with red blood cells. No multinuclear or large vesicular nucleated cells are found.

Pancreas.—The gland is apparently normal.

Mesenteric Lymph Glands.—Capsule shows moderate thickening with extensive round cell infiltration. The sinuses are in areas entirely obliterated by the proliferative and desquamative endothelial cells of the lymph spaces. Many of the lymphoid follicles have been entirely replaced by a necrotic tissue surrounded and infiltrated by large epithelioid, round cells and giant cells of the bipolar and mural type. Scattered within the tissue are polymorphonuclear leucocytes, especially seen around the areas of necrosis. Many of the germinal follicles seem to be the starting point for the tuberculous process. No bone marrow giant cells or vesicular nucleated cells (except the epithelioid cells) are seen.

Bone Marrow.—There is an enormous hyperplasia of the cellular and connective tissue elements. The types of cells seen are of all the varieties seen in the bone marrow under normal conditions (Fig. 3). Thus the types seen are, neutrophilic and eosinophilic myelocytes, polynuclear leucocytes, giant cells, large and small mononuclear cells, normocytes, normoblasts and megoblasts, together with large numbers of fibroblasts of the connective tissue. The cells as a whole lie embedded in a very much thickened and well-formed connective tissue membrane containing well-developed blood vessels and capillaries (Mallory's connective tissue stain). The neutrophilic myelocytes are for the most part arranged in clusters and are occasionally seen, bunched together in spaces lined with endothelium. The cells described as myelocytes are the fairly large mononuclear cells which appear to contain neutrophilic and eosinophilic granules; no true basophilic myelocytes are seen. Scattered indiscriminately throughout the tissue are large giant cells which appear in two forms (though their nuclei which often contains a nucleolus and their nuclear fibrillar network together with their hyaline appearing protoplasm, are the same) : one type of cell possesses numerous nuclei, arranged generally in the center, and is composed of large vesicular nuclei and nucleoli with a well-defined network and surrounded by a hyaline appearing protoplasm. To this type is applied the term polykaryocyte; the other type or megokaryocyte possesses but one singular lobulated nuclear cast, possessing a nucleolus, well-defined nuclear membrane and surrounded by a large amount of hyaline appearing protoplasm. Throughout the section are noted enormous numbers of very large cells with vesicular nucleus about which is a small, apparently non-granular, rim of protoplasm, which has a tendency toward taking the alkaline stain. These cells contain often a well-developed nucleolus. They have a tendency toward cluster arrangement, and some definite masses of these cells are seen in spaces lined by endothelial cells. Arranged among erythrocytes and also scattered indiscriminately among the other cells are fairly large numbers of normoblasts, some of whose nuclei are in the process of extrusion. An occasional megoblast with dividing nucleus may be seen. The polynuclear cells are scarce as compared with those of normal bone marrow, though here and there are noted some. They have no tendency toward arrangement in clusters. There are definite large masses of small mononuclear cells or lymphocytes seen in different portions of the sections. An occasional polynuclear eosinophile with no definite position is seen. Coursing everywhere are enormous numbers of fibroblasts and larger and more developed connective tissue cells. The blood vessels are large and well developed with thickened walls. The capillaries are markedly increased also. There are no organisms seen in sections. A

noticeable feature is the presence of very large numbers of small mononuclear cells and fibroblasts with a relative decrease in the normal functionatory marrow cells.

Spleen.—Capsule is somewhat thickened, is infiltrated with round cells and has attached to it small and large strands of connective tissue. The trabeculæ and blood vessels show a slight hyperplasia; there is a marked increase in the reticulum. Many of the Malpighian bodies appear intact though the majority of them show some atrophy, many of them having disappeared. The splenic pulp is markedly hypertrophied and is found to contain large clusters of well-formed and well-preserved red blood corpuscles. Scattered here and there among the pulp cells are seen very large cells with vesicular nuclei, some cells containing but a rim of a homogeneous protoplasm, while in others the protoplasm is more extensive and apparently contains what are perhaps neutrophilic granules. There is a marked tendency on the part of these cells to cluster together and lie within spaces lined by epithelial cells. An occasional multinucleated giant cell of the bone marrow type is seen, many normoblasts are visible. There is an extraordinarily small amount of pigment.

Splenic Nodes.—Surrounded by a tissue which microscopically resembles the above are areas from 1 to 2 cm. in diameter, which greatly differ in some respects from the surrounding tissue. These areas (Fig. 1) are seen to be made up almost entirely of large cells, containing a vesicular nucleus and but a rim of protoplasm. They for the most part are seen in small spaces, some of which appear to be lined with endothelial cells. Bone marrow giant cells, both of the multinuclear and polynuclear forms, are seen in abundance (Fig. 2). All these cells are supported by a connective tissue meshwork, which contains also large numbers of nucleated red cells and erythrocytes arranged in no particular fashion. No pigment is seen.

Microscopic Diagnosis.—Œdema and congestion of lungs. Fibrinous pleurisy. Chronic interstitial myocarditis. Fatty metamorphosis of heart and liver. Chronic splenic tumor, with bone marrow cell hyperplasia. Cloudy swelling of heart, liver and kidneys. Chronic diffuse nephritis. Primary tuberculosis of mesenteric lymph nodes. Sclerosis of bone marrow.

As the term splenomegaly is now applied it has especial reference to a splenic hypertrophy which has extended over a period of months, perhaps years. Some writers include under the word splenomegaly only those conditions in which there is an idiopathic enlargement of the spleen, accompanied by an anæmia or perhaps an anæmia with a cirrhotic liver. Others apply the term to a condition in which there is an enlarged spleen with known or unknown etiology. The causes of many of the splenomegalies is still so uncertain that to formulate any definite classification from an etiological standpoint is wholly impossible. It is not the purpose of this report to discuss all of the various conditions which may give rise to an enlarged spleen. The main interest arises as to the

etiology of these cases grouped under the term "splenic anæmia." At the present time their origin is attributed to some intoxication, chronic in form, which principally affects the spleen, though sclerosis of the splenic and portal vessels and thrombosis of the splenic sinuses have been given as possible causative agents. That a diseased bone marrow might be the contributing cause in some of the cases has not been suggested, and it is important, therefore, to present an example in which the bone marrow is as extensively affected as the spleen.

This appears to be the condition in the case just described. What toxin, if any, caused the chronic inflammation of the bone-marrow in this case is not known. It should be remembered, too, that from the histological examination the bone-marrow presents a more intense and, perhaps, more advanced sclerosis than does the spleen, and that the greater part of the bone-marrow elements, as seen in the sections, are not the true blood-forming cells of the body, but are principally lymphoid cells and fibroblasts. Associated with this intense sclerosis of the bone-marrow are the definite foci composed of true bone-marrow elements in the spleen.

It is needless to add that a differential diagnosis from the other splenomegalies is not necessary. From a review of the literature it has been seen that the case described above does not resemble the types heretofore reported.

The pathological changes which take place in the various forms of splenomegaly, particularly of the primary types, are not uniform and the relative bearing of the bone-marrow upon cases of splenomegaly has been but briefly commented upon. It is, therefore, only by careful examination of individual cases and by experimentation upon animals that we may hope to arrive, some day, at conclusions as regards the physiologic and pathologic properties of the splenic hypertrophies.

In consideration of all cases of enlarged spleen, especially when the etiology is entirely unknown, we must of necessity turn to the so-called hæmopoëtic organs in which group are included, besides the spleen, the bone-marrow, lymph glands, liver and hæmolymph glands. That a decided relationship exists between the first three has been proved by all who have spent much time in study of the

subject. The liver too undoubtedly plays an important rôle, while Warthin's (1) work on the "Anatomy and Physiology of the Hæmolymph Glands," together with W. B. Drummond's (2) monograph on the same subject, have brought these structures into an important relationship with the other tissues considered as exciting some special action on the blood's function or destruction. Bizzozero and Neuman, long ago, demonstrated the fact that the bone-marrow was the seat of formation of the red blood cells. Soon after Ehrlich not only verified this statement, but also demonstrated that the bone-marrow was the site of white cell formation. Investigators of later date have found these facts to be true, and we are now in the position to give the most important functions of the bone-marrow. The function of the lymph gland cannot be asserted as definitely, but the fact remains that the lymph glands cannot be excluded from those organs described as being hæmopoëtic. They are possibly the place of origin of the lymphocytes of the circulating blood and from chemical and experimental observations it appears that they have power to assume, at some time, perhaps a compensatory function. The liver in embryonal life aids in the blood's formation, while as age advances it gradually loses that power and helps in the carrying off of waste matter, the result of disintegration of red cells. The functions of the hæmolymph glands are still in dispute; thus Drummond (2) claims that "there is no sufficient evidence that the hæmolymph glands play any part in the formation of red blood cells, but that on the other hand they appear to perform a very active part in the destruction of red blood cells and in the liberation of pigment." On the other hand, Warthin (1) says that "the close relations between spleen, lymph glands and bone-marrow is shown by the power of the hæmolymph glands to take on a structure of either spleen or marrow and to compensate for these organs when their function is abridged by disease." And finally the spleen has perhaps caused more discussion than any of the other organs. Differences in the cellular contents and structural characters in various animals has proven a barrier for decisive experimental work. Thus in lower vertebrates and in some of the mammalia, as the mouse, hedgehog and rabbit, nucleated red cells are normally present, giving evidence of a formation of red blood

cells in the spleen. Ehrlich (3), on the other hand, states that in the human spleen nucleated red blood cells are not to be found normally. Other investigators do not substantiate this phase of Ehrlich's work and claim that under normal conditions normoblasts are often found in the human splenic pulp. It is for this reason extremely difficult in animal experiments to determine the part taken by the spleen in forming erythrocytes. It is generally believed that though the spleen may be one of the sources of origin of red blood cells, its main duty is the absorption of the material due to disintegration of red and white cells and to preserve a portion of it, at least, for the organism.

The main point of interest which concerns us is the significance that may be attached to a pathological change in the spleen in which we see definite bone-marrow elements, including giant cells arranged in isolated areas through the splenic pulp. The presence of giant cells, resembling in all respects those of the bone-marrow existing in the human spleen, has been mentioned and but slightly commented upon by Dock and Warthin (4), Rolleston (5) and Simonds (6). Dock and Warthin interpret them as being emboli, while Rolleston and Simonds offer no hypothesis. On the other hand, numerous investigators, such as Dominici, Opie, Jarotsky, Bunting and others have commented upon their presence in the hedgehog, the rabbit and the guinea pig, under normal as well as under pathological conditions. Under all normal conditions bone-marrow giant cells are absent from the human spleen. It is only during an acute or chronic intoxication that they appear in the human spleen. I have not been able to find a note on their presence in the acute infections of human beings, though in one case which I have studied the spleen from a patient dying with acute gonorrhœal endocarditis, showed definite bone-marrow cells, including the true myeloplaxes and the large granuleless mononuclears arranged in small clumps. Dock and Warthin's report on the pathology of two cases of " splenic anæmia " is interesting since in one of their cases giant cells resembling those of the bone-marrow were found in great numbers in the lung capillaries, while a smaller number were found in the liver capillaries and blood spaces of the spleen and hæmolymph glands. In the other case there were found large

numbers of these cells in the lungs, a smaller number in the spleen and a fair number in the hæmolymph glands. In both cases there was a decided decrease of giant cells in the bone marrow. As stated above, the writers considered these cells emboli.

The question now arises whether there are splenomegalies in which the bone-marrow's function has become exhausted by the action of some circulating toxin and in which the spleen exerts itself in a compensatory manner by forming new blood elements for the organism.

Osler, Sippey (7) and Simonds (6) have thoroughly reviewed the literature of splenomegaly and hence the citation of cases will be dwelt upon only so far as it may have a definite bearing on the subject under discussion. The cases of so-called " splenic anæmia " or splenic anæmia with cirrhosis of the liver (Banti's disease) form the greater part of the cases of obscure origin. In these the pathology has been quite well outlined by Rolleston (5), who says that the main pathologic changes in splenic anæmia are: (1) Fibrosis of the organ, (2) atrophy of the lymphoid elements and (3) fibrosis of the Malpighian bodies. In addition there is usually no evidence of exaggerated hæmolysis such as excessive pigmentation of the spleen. He notes also that there is usually a red marrow transformation, though the yellow marrow may persist.

The pathological changes seen in the present case are entirely different from those which have been described previously. Macroscopically distinct small, rather soft areas, of a light red color, spherical in form, varying in size from a pin head to that of a large bean, were quite striking. Simonds (6) describes an enlarged spleen as containing " numerous round, oval or branched reddish brown areas about two millimeters in diameter." In this case the areas, containing a large amount of pigment, give an intense iron reaction. The difference is obvious, since the areas described in my case are free from pigment and are seen to be made up of collections of what appear to be definite bone-marrow cells. There was great similarity between sections of the bone-marrow and sections through these areas in the spleen. For a comparative study of the bone-marrow and the cells in the nodules the most useful stains were Duval's modification of Leishman and Wright stains,

polychrome methylene blue, hæmatoxylin and eosin, Mallory's connective tissue stain, Van Giesen's stains and Ehrlich's triacid stain. With none of these stains, however, have definite neutrophilic granules been made out in the cells, either in the spleen or bone-marrow. This is perhaps due to the fixation which unfortunately was entirely in Zenker's fluid. Some of the cells of the bone-marrow presented, what appeared to be, neutrophilic granules. There were cells in the spleen which had the same appearance, but I cannot state positively whether they were or were not myelocytes. It can be definitely said, however, that the giant cells, the megakaryocytes, were present in both tissues and that the large granuleless mononuclear cells found normally in the bone-marrow alone were present in great abundance in these nodules in the spleen.

The question now arises whether this enlargement of the spleen should be considered as an heteroplastic growth or as an hyperplastic. We meet with three hypotheses: (1) That the condition is due to a disease of the spleen *per se,* (2) that the bone-marrow foci found in the spleen are emboli and the enlargement due to an intense congestion, (3) that the enlargement depends upon the spleen assuming a dormant hæmopoëtic function with hyperplasia of the cells of the spleen pulp.

The exact determination as to whether the condition was primary in the spleen is impossible. From the examination of the bone-marrow it seems more probable that the sclerosis there is of an older form and of a greater intensity than it is in the splenic pulp. The theory that these masses of bone-marrow tissue arise from bone-marrow cell emboli cannot hastily be disposed of, for the two cases reported by Dock and Warthin, referred to above, which showed definite bone-marrow cells in the capillaries of the liver, spleen, hæmolymph glands and lungs, together with Bunting's (10) report of the finding of definite bone-marrow in the dorsal portion of the aorta, would perhaps suggest the possibility of such an emigration of cells. Bunting, however, states that one is not justified in deciding whether they were formed by a further metaplasia of connective tissue cells or by a metaplasia of emigrated cells from the blood stream, capable of differentiation into the various types of marrow cells. In my case it does not seem probable that the

bone-marrow elements have been brought to the spleen by the blood stream, for if such were the case it would seem reasonable to suppose that these same cells should be found in the lungs and liver, as in the two cases of Dock and Warthin. On the other hand, possibly such a process cannot be entirely ruled out for the clusters of marrow cells, some of which are found in blood spaces, suggest at least the idea that they may be emboli. However, from a careful study of the sections as regards the position of the bone-marrow cells and the condition of the lymph nodes, liver and lungs, I do not feel justified in stating that they are brought directly to the spleen from the bone-marrow.

Finally we come to the third proposition as to whether the enlargement may be due to the development within the spleen of blood-forming elements. This hypothesis really rests upon two conditions, namely: (1) that the bone-marrow cells are fœtal remains of cells which have been lying latent and through a stimulant, perhaps exciting the bone-marrow to activity and finally to uselessness, have increased in proportion and have taken on their original fœtal blood-forming function; (2) that the spleen has produced bone-marrow cells from the blood or lymph spaces. Before arriving at any conclusion whatsoever as regards the logic of either of these two hypotheses, it is necessary to consider the subject, as best we can, from an experimental standpoint, for it is only by this means that we may throw some light on the subject. Indeed from the sections of organs removed at autopsy no consecutive changes can be noted.

It is an accepted fact that the human and mammalian fœtal spleens contain a varying number of bone-marrow cells such as the giant and myelocytic cell together with large numbers of nucleated red blood corpuscles. As the human spleen becomes further developed the bone-marrow and normoblastic cells gradually disappear while in the spleens of many of the mammals the blood foci cells persist through life though in greatly decreased numbers.

Experiments of Pugliese (11) on hedgehogs have shown that after repeated blood lettings the giant cells increase markedly in the spleen, and that after partial splenectomy the giant cells, in the intact splenic tissue, show a decided increase; that with total extir-

pation of the spleen the bone-marrow becomes filled with giant cells and that after repeated venesections the giant cells increase in the spleen but remain unchanged in bone-marrow, whereas in the liver and lymph glands there are no traces of them. The experimental anæmias produced by Bunting (12) in rabbits throw much light on the compensatory power of the spleen. Thus after sclerosis of the bone-marrow had been produced the spleen showed a marked dilatation of the peripheral venous sinuses which were crowded with marrow cells, and especially numerous were the megakaryocytes. Myer and Heincke (13) have shown similar changes in the spleen in a case of pernicious anæmia. Bunting (12) comments upon the fact that bone-marrow, following injection of hæmolytic toxins, becomes sclerotic and there may be an almost entire replacement of the hæmopoëtic elements by the newly formed connective tissue, while hæmorrhages cause only the hyperplastic marrow of a secondary anæmia. Clinically it has been noticed that an enlargement of the spleen often causes an improvement in the general condition of the bloods.

Dominice (14) has subjected rabbits to repeated bleedings and has found practically the same histological changes in the spleen as has Bunting. He has observed, however, that in the course of experimental infections with the typhoid bacillus there takes place what may be called "a partial myeloid change." By this term Dominice means that all the elements of the bone-marrow are not present. Thus there are noted large normoblasts and basophilic and neutrophilic myelocytes, but the eosinophilic myelocytes and giant cells are very rare or entirely absent. Jarotsky's (15) experiments with white mice infected with the hog cholera bacillus further substantiates the findings of Bunting, Pugliese, Dominice and others. Jarotsky produced in twenty-four hours an enormous increase of giant cells and myelocytes in the spleen.

Opie (19) has observed the occurrence of eosinophilic myelocytes and bone-marrow giant cells, in the spleens of guinea-pigs four hours after inoculation with Bacillus pyocyaneus, Bacillus anthracis, and Bacillus mucosus capsulatus—he believes that these elements are derived from the bone-marrow and are not formed in the spleen.

With the exception of some of Bunting's work on the experi-

mental anæmias, investigations of the subject have been limited to the acute infections. It is indeed fortunate that cases have been recorded in which similar splenic changes have taken place in the course of what appears in the present state of our knowledge to be a condition of chronic intoxication or infection, namely pernicious anæmia. Careful histological study of seventeen cases of pernicious anæmia by Gulland and Goodall (16) shows that in eight of the seventeen cases cells like those of the bone-marrow were found in the liver, while in four cases myelocytes were found in the spleen, and in one case there were typical large bone-marrow giant cells in the spleen.

Dominice and Jarotsky have written at length upon the possible mode of origin of the bone-marrow cells in the spleen. Their findings, though interesting, are not at all convincing. The theories of transportation of bone-marrow cell to the various organs can be substantially upheld by anatomical evidence in the leukæmias. That the phenomenon takes place, however, in all cases, where cells resembling those of the bone-marrow are found in the various organs, cannot be supported. On the other hand, experimental and anatomical evidence go to substantiate, if not prove, the theory that the spleen under certain conditions of chronic inflammation of the bone-marrow may take up the hæmopoëtic function. What the toxin is cannot be proven in many cases; but whatever the chemical changes may be, the toxin must have some deleterious effect upon the bone-marrow, either prohibiting the growth of cells or so stimulating them as to finally exhaust their power to produce blood cells. It is but natural to suppose that the spleen lying dormant, as it were, in the body should at times be called upon to perform or at least aid in performing the activities of the marrow.

CONCLUSIONS.

1. The nodules found in the spleen are islands of active hæmatoplastic tissue.

2. The bone-marrow, at least in the case which has been described, has been the primary focus of disease; some toxin has probably produced a chronic inflammatory change.

3. The bone-marrow, owing to its enormous sclerosis, has lost

its hæmopoëtic powers totally or to a marked degree and the spleen has reverted to its fœtal power to form blood.

BIBLIOGRAPHY.

1. Warthin, *Jour. of Med. Research,* 1901, vi, 13.
2. Drummond, *Jour. of Anat. and Physiol.,* 1899–1900, xxxiv, 198.
3. Ehrlich and Lazarus, Histology of Blood, Cambridge University Press, 1900, p. 99.
4. Dock and Warthin, *American Jour. of the Med. Sciences,* 1904, xxvii, 25.
5. Rolleston, *British Med. Jour.,* 1903, ii, 573.
6. Simonds, *Jour. of Infectious Diseases,* 1908, v, 23.
7. Sippey, *American Jour. of the Med. Sciences,* 1899, cxviii, 570.
8. Bovaird, *American Jour. of the Med. Sciences,* 1900, cxx, 377.
9. Sippey, *American Jour. of the Med. Sciences,* 1899, cxviii, 570.
10. Bunting, *Jour. of Exper. Med.,* 1906, vii, 365.
11. Pugliese, *Fortshr. der Med.,* 1897, xv, 727.
12. Bunting, *Jour. of Exper. Med.,* 1906, viii, 625.
13. Meyer and Heincke, *Verhandl. der deutschen pathol. Gesel.,* 1906, ix, 224.
14. Dominice, *Arch. de méd. expér. et d'anat. path.,* 1901, xiii, 1.
15. Jarotsky, *Virchow's Arch.,* 1908, cxci, 112.
16. Gulland and Goodall, *Jour. of Path. and Bact.,* 1905, x, 125.
17. Weber, *British Med. Jour.,* 1904, i, 1416.
18. Bunting, *Johns Hopkins Hosp. Bull.,* 1905, xvi, 222.
19. Opie, *Am. Jour. Med. Sciences,* 1904, cxxvii, 988.

EXPLANATION OF PLATES XXXII AND XXXIII.

FIG. 1. Section from localized nodule in spleen (low magnification).
FIG. 2. Section from nodule in spleen (high magnification).
FIG. 3. Section from bone-marrow.

FIG. 1.

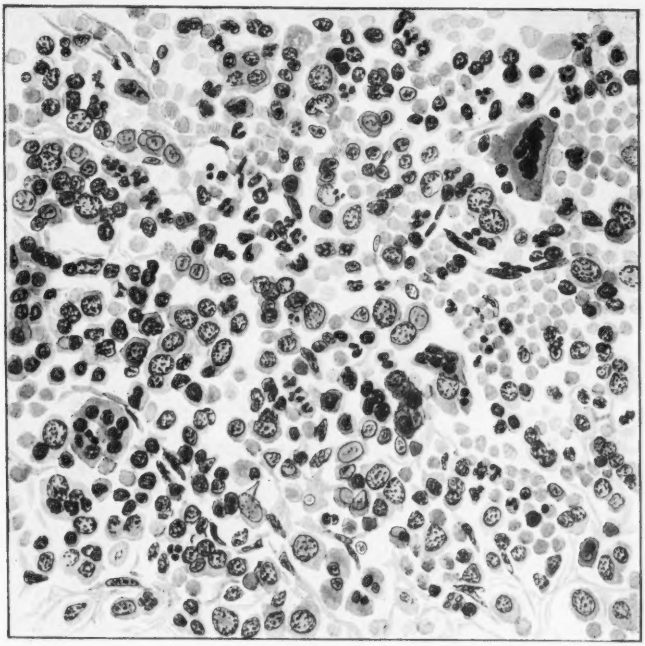


FIG. 2.

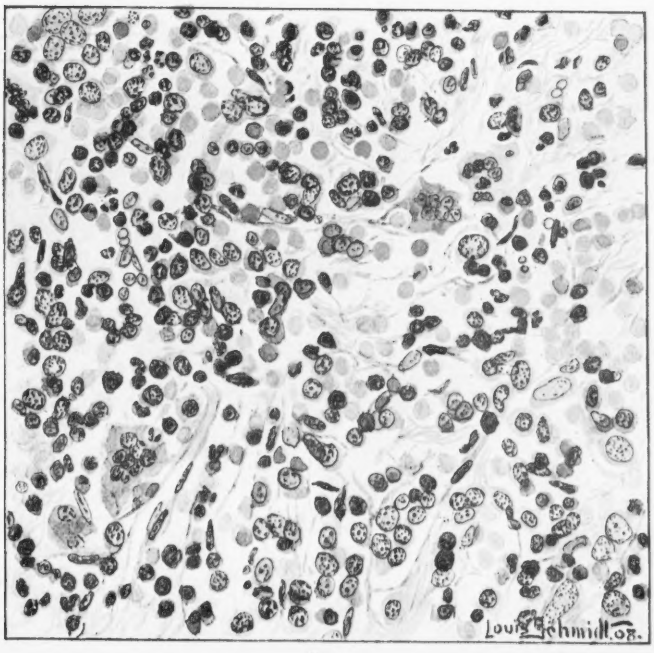


FIG. 3.